

Renal Dysfunction in Liver Disease



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To Granny

ABSTRACT

Renal dysfunction is a major cause of morbidity and mortality in hepatology patients. In cirrhosis, portal hypertension-related renal dysfunction evolves in parallel with advancing disease, and has important prognostic implications. Similarly, in acute liver failure, acute kidney injury is associated with increased mortality and may impact on distant organ function by driving cardiac, lung, brain, as well as liver injury. Liver transplantation is the definitive treatment for portal hypertension-related renal dysfunction in cirrhosis and acute kidney injury in acute liver failure. Yet, liver transplantation itself is complicated by both acute kidney injury and chronic kidney disease. Despite the frequency of occurrence and devastating consequences of renal dysfunction in liver disease, treatment options remain limited and there is a desperate need for advancement of scientific understanding. In this thesis I have studied 3 main aspects of renal dysfunction in liver disease.

Firstly, I examined the systemic haemodynamic and renal effects of acute endothelin-1 receptor antagonism in patients with advanced portal hypertension-related renal dysfunction. In a randomised, double-blind, placebo controlled, crossover study of patients with refractory ascites acute combined endothelin-A and endothelin-B receptor antagonism caused a fall in glomerular filtration rate despite no change in systemic haemodynamics or total renal blood flow, and a marked reduction in urinary flow rate. These findings are consistent with a reno-protective role for endothelin-1 in portal hypertension-related renal dysfunction.

Secondly, I explored the hypothesis that the acute renal dysfunction that occurs in acute liver failure is distinct from the hepatorenal syndrome of cirrhosis, and instead the systemic inflammatory response may be a critical determinant. I demonstrated that the systemic inflammatory response syndrome is associated with acute kidney injury in acute liver failure patients. Importantly, this relationship was independent of the presence of infection and of severity of liver injury. Thereafter, in patients super-urgently transplanted for acute liver failure I found that, in contrast to patients undergoing elective liver transplantation, pre-transplant acute kidney injury was not associated with the development of chronic kidney disease. The results support an alternative pathophysiological process underlying the renal injury that occurs in acute liver failure.

Finally, I examined the long-term decline in renal function and progression to chronic kidney disease in liver transplant recipients. I observed that patients have a clinically relevant decline in glomerular filtration rate beyond the initial post-operative period, and current focus of chronic kidney disease prevention. Multivariate modelling identified several potentially modifiable patient factors associated with a faster rate of decline.

The studies presented have helped to further our knowledge of portal hypertension-related renal dysfunction, acute kidney injury in acute liver failure and chronic kidney disease after liver transplantation. By doing so, we have moved one step closer to improving patient morbidity and mortality as a result of renal dysfunction in liver disease.

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DECLARATION

This thesis represents research undertaken in the Centre for Liver and Digestive Disorders in the Royal Infirmary of Edinburgh, in collaboration with the Clinical Pharmacology Unit University of Edinburgh and the Centre for Cardiovascular Science University of Edinburgh. Financial support was provided by a Wellcome Trust Grant and a Lothian NHS Trust research fund. The studies were undertaken with support of the Local Research Ethics Committee or Royal Infirmary of Edinburgh Audit Department and in accordance with the Declaration of Helsinki. Chapters 3, 4, 5 and 6 have been published in peer-reviewed journals.

I declare that all the work presented is my own except where stated below, and it has been entirely composed by myself. This thesis has not been submitted for any other degree, postgraduate diploma or professional qualification.

Studies

Chapter 2: Protocol design was by Dr J Leithead, Dr J Ferguson, Dr Prof P Hayes, Prof D Webb, Dr J Goddard, and Prof D Newby. The study was performed by Dr J Leithead and nurses in the Wellcome Trust Clinical Research Facility, particularly Mrs F Paterson and Mrs J Antonelli. Healthy control data was provided by Dr J Goddard from her study of endothelin-1 receptor antagonism in health and chronic kidney disease.

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ABBREVIATIONS

ALF = acute liver failure

ALT = alanine aminotransferase

AKI = acute kidney injury

BMI = body mass index

CARS = compensatory anti-inflammatory response syndrome

CI = confidence interval

CKD = chronic kidney disease

CLD = chronic liver disease

CNI = calcineurin inhibitor

CO₂ = carbon dioxide

CrCl = measured creatinine clearance

c-statistic = concordance statistic

DAMP = damage-associated molecular pattern

ECE = endothelin-converting enzyme

EFF = effective filtration fraction

eGFR = estimated glomerular filtration rate

eGFR(MDRD4) = estimated glomerular filtration rate derived from 4-variable MDRD equation

eGFR(MDRD5) = estimated glomerular filtration rate derived from 5-variable MDRD equation

eGFR(MDRD6) = estimated glomerular filtration rate derived from 6-variable MDRD equation

eNOS = endothelial nitric oxide synthase

ERBF = effective renal blood flow

ERPF = effective renal plasma flow

ERVR = effective renal vascular resistance

ET-1 = endothelin-1

ET-A = endothelin-A

ET-B = endothelin-B

G-CSF = granulocyte colony-stimulating factor

GFR = glomerular filtration rate

ΔGFR = mean annualised change in eGFR from 6 months to 5 years post transplant

H⁺ = hydrogen ion

HCO_3^- = bicarbonate
 Hct = haematocrit
 HMGB1 = high-mobility group box-1 protein
 HR = hazard ratio
 ICP = intracranial pressure
 IL = interleukin
 In = inulin
 iNOS = nitric oxide synthase
 INR = international normalised ratio
 IQR = interquartile range
 KCH = Kings College Hospital
 LBP = lipopolysaccharide binding protein
 LD = low dose
 logeGFR(MDRD4) = log eGFR calculated using the MDRD 4-variable equation
 logeGFR(MDRD5) = log eGFR calculated using the MDRD 5-variable equation
 logeGFR(MDRD6) = log eGFR calculated using the MDRD 6-variable equation
 MAP = mean arterial pressure
 MARS = molecular adsorbent recirculating system
 MDRD = Modification of Diet in Renal Disease
 MELD = Model for End Stage Liver Disease
 MELD(adj) = MELD score with regression coefficients adjusted for our model
 MELD(CrCl) = MELD score with logeCrCl substituted for logecreatinine
 MELD(eGFR) = MELD score with logeGFR substituted for logecreatinine
 MMF = mycophenolate mofetil
 OA = on admission to hospital
 OR = odds ratio
 PAH = para-aminohippurate sodium
 PRA = plasma renin activity
 PRR = pattern recognition receptor
 RAAS = renin-angiotensin-aldosterone system
 RIFLE criteria = acronym indicating Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal disease
 ROC = receiver-operating characteristic
 RRT = renal replacement therapy
 SD = standard deviation

SEM = standard error of the mean

SIRS = systemic inflammatory response syndrome

SNS = sympathetic nervous system

STD = standard dose

SVRI = systemic vascular resistance index

TIPSS = transjugular intrahepatic portosystemic stent-shunt

TLR = Toll-like receptor

TNF = tumour necrosis factor

TNF- α = tumour necrosis factor-alpha

UFR = urinary flow rate

UKELD = UK score for Patients with End-Stage Liver Disease

UKELD(adj) = UKELD score with regression coefficients adjusted for our model

UKELD(CrCl) = UKELD score with logeCrCl substituted for logecreatinine

UKELD(eGFR) = UKELD score with logeGFR substituted for logecreatinine

WCC = white cell count

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CHAPTER 1

An introduction to renal dysfunction in liver disease

1.1 Introduction

Renal dysfunction is a major cause of morbidity and mortality in hepatology patients. In cirrhosis portal hypertension-related renal dysfunction evolves in parallel with advancing disease, and has important prognostic implications. Similarly, in acute liver failure (ALF), acute kidney injury (AKI) is associated with increased mortality and may have impact on distant organ function by driving cardiac, lung, brain, as well as liver injury. Liver transplantation is the definitive treatment for portal hypertension-related renal dysfunction in cirrhosis and AKI in ALF. Yet, liver transplantation itself is complicated by both AKI and chronic kidney disease (CKD). Despite the frequency of occurrence and devastating consequences of renal dysfunction in liver disease, treatment options remain limited and there is a desperate need for advancement of scientific understanding in this field.

1.2 Chronic liver disease: The spectrum of portal hypertension-related renal dysfunction

In the UK chronic liver disease (CLD) is the only major cause of death that continues to rise, being the 5th 'big killer' in England and Wales (1). In Scotland specifically cirrhosis mortality rates have more than doubled in the last 20 years and are now amongst the highest in Western Europe (2,3).

Renal dysfunction is a common complication of CLD that has significant implications for patient morbidity and mortality. Portal hypertension, and the resulting circulatory and neuro-humoral derangement, is associated with a progressive functional renal impairment that evolves in parallel with advancing disease (4). This spectrum ranges from tubular dysfunction to hepatorenal syndrome, and first becomes evident in early, compensated cirrhosis (4). Multiple additional triggers may exacerbate the physiological changes of portal hypertension, including large volume paracentesis, gastro-intestinal haemorrhage and diuretic induced intra-vascular volume depletion (5,6). Moreover, intrinsic renal disease is relatively frequent reflecting the prevalence of co-morbidity such as diabetes mellitus and hypertension, and glomerulonephritides associated with alcoholic liver disease, hepatitis B and hepatitis C (7,8).

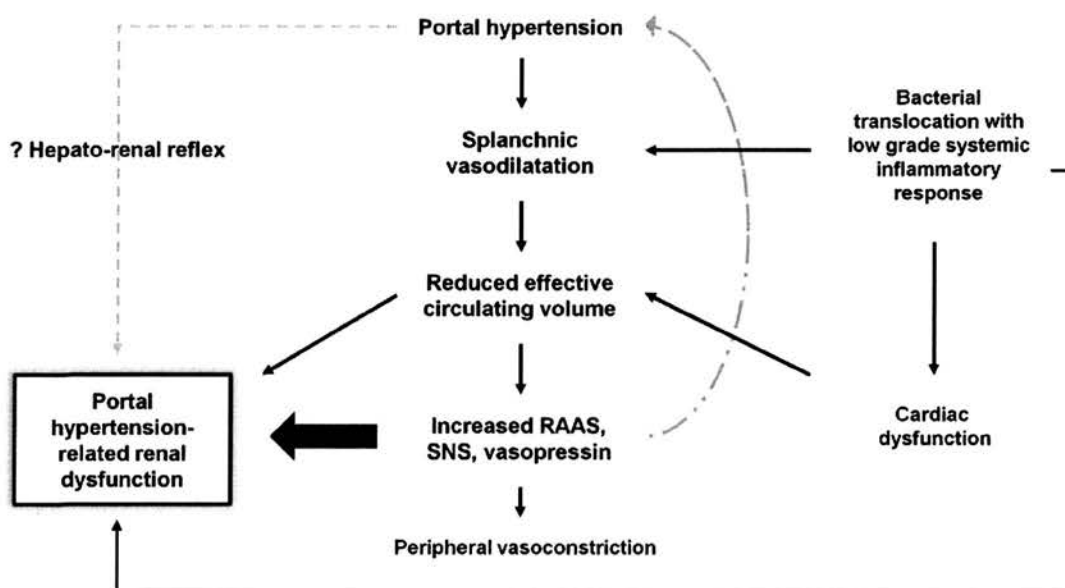
The aetiology of renal dysfunction in patients with CLD has important ramifications for prognosis. In portal hypertension-related renal dysfunction the glomerular filtration rate (GFR) only falls appreciably once disease is advanced and circulatory

derangement severe (4). Consequently, the 3-month probability of survival for hospitalised patients with hepatorenal syndrome is only 15% compared with 73% for those with parenchymal nephropathy (5). Despite evolving understanding of the pathogenetic mechanisms of portal hypertension-related renal dysfunction treatment options remain limited.

The systemic circulation in chronic liver disease

The currently accepted theory that best explains the systemic circulatory derangement that accompanies portal hypertension is based on the Peripheral Arterial Vasodilatation Hypothesis, published in 1988 (Figure 1.1) (9). This suggested that systemic vasodilatation and relative intravascular underfilling is the driving force, with the subsequent activation of multiple homeostatic neurohumoral pressor systems in an effort to maintain the effective circulating volume. More recently it has become apparent that the vasodilatation is primarily limited to the splanchnic vascular bed (10). In contrast, blood flow within the renal vessels as well as those that supply the brain, muscles and skin, is reduced (11,12,13). Thus, peripheral vasoconstriction probably occurs as a result of the increased pressor activity and is a compensatory response to progressive splanchnic vasodilatation (14).

Figure 1.1: Systemic circulatory derangement that accompanies portal hypertension: Progressive splanchnic vasodilatation and a reduced effective circulating volume stimulate increased RAAS, SNS, and vasopressin activity, the primary mediators of renal tubular and haemodynamic dysfunction. In advanced disease, a systemic inflammatory response and cardiac dysfunction play a contributory role.



Abbreviations: RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system.

- Splanchnic vasodilatation

Many vasoactive agents have been proposed as mediators of the vasodilatation of the splanchnic vasculature. However, nitric oxide is considered to be of central importance in both instigation and potentiation (15,16,17,18). In early portal hypertension, vascular stretch within the intestinal microcirculation may trigger endothelial nitric oxide synthase (eNOS) upregulation, and it is speculated that this is the first step in the neurohumoral and circulatory cascade (19,20). In the hyperdynamic phase, shear stress, endotoxaemia and vasoactive mediators such as vascular endothelial growth factor (VEGF), oestrogen and bradykinin have all been implicated in the increased generation of eNOS (21,22,23,24,25,26,27,28). Inducible nitric oxide synthase (iNOS) may contribute to the greater nitric oxide activity in this later stage. Although studies in rats have failed to consistently demonstrate upregulation of splanchnic or systemic iNOS, the endotoxaemia and hypersecretion of inflammatory cytokines that is often displayed by cirrhotic patients are known stimuli of iNOS synthesis (25,28,29,30,31,32). Furthermore, specific iNOS inhibition in the forearm of cirrhotics suggests that this isoform may be involved in the regulation of at least peripheral vascular tone (33).

Despite these findings, it is clear that nitric oxide is not the sole mediator of the splanchnic vasodilatation. eNOS/iNOS knockout mice develop circulatory dysfunction following portal vein ligation, and chronic nitric oxide inhibition in cirrhotic rats delays but does not prevent the vasodilatation of the splanchnic vascular bed (34,35). *Angeli et al* demonstrated that while nitric oxide may be

significant in the early phase its role in maintenance may be partial at most (36). Other vasodilating agents have therefore been implicated including glucagon, prostacyclin and calcitonin-gene related peptide (37,38,39).

- Compensatory peripheral vasoconstriction

With progressive splanchnic vasodilatation and intravascular underfilling, high pressure baroreceptors within the aorta, carotid sinus and juxtaglomerular apparatus of the kidney activate the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS), and stimulate non-osmotic hypersecretion of vasopressin (Figure 1.1) (40,41,42,43). Arterial blood pressure becomes critically dependent on these pressor agents (44,45,46). However, although peripheral vasoconstriction ensues, the splanchnic vasodilatation continues to progress due to an imbalance between vasoactive mediators and a possible vascular hyporesponsiveness to pressor agents (47,48,49,50,51,52). The circulation becomes increasingly hyperdynamic with patients displaying the typical clinical picture of reduced systemic vascular resistance, increased heart rate and cardiac output, expanded blood volume, and eventually arterial hypotension (53,54,55).

The spectrum of portal hypertension-related renal dysfunction

The functional renal impairment of portal hypertension is characterised by increased tubular sodium reabsorption, impaired free water clearance, renal vasoconstriction and pre-renal azotemia (4). This spectrum first becomes evident in early, compensated cirrhosis, and progresses in parallel with advancing disease. The true incidence of the various stages of renal dysfunction is unclear. However, in one study the 5-year probability of the development of ascites and hepatorenal syndrome after presenting with variceal haemorrhage was 73% and 21%, respectively (56).

The RAAS, SNS, vasopressin and possibly endothelin-1 (ET-1) are recognised amongst the principle mediators of the renal tubular and haemodynamic dysfunction that accompanies portal hypertension (4). Importantly, these systems have intertwining positive regulatory effects (57,58,59,60). For example, renal β -adrenoceptor activation stimulates secretion of renin from the juxtaglomerular apparatus, whilst angiotensin II in turn enhances the release of noradrenaline (57). Such complex interactions further exacerbate the neurohumoral overactivity of the portal hypertensive syndrome.

- Exaggerated renal tubular sodium reabsorption

Renal sodium retention plays a central role in ascites formation and peripheral oedema in patients with cirrhosis (4). A gradual deterioration in renal tubular function is observed that begins in the compensated state. Pre-ascitic cirrhotic

patients demonstrate impaired renal sodium metabolism, with reduced natriuresis in the standing position and following a saline load (61,62). With disease progression sodium retention becomes overt, there is a positive sodium balance, and ascites develops. The onset of refractory ascites is accompanied by profound sodium retention, with patients excreting less than 10 mmol of sodium per day (63). In early stages, renal blood flow and GFR remain within normal limits and sodium retention occurs solely at the tubular level (9,64). Later, once the GFR drops, reduced filtered sodium contributes and exacerbates sodium retention (4).

Patients with ascites frequently demonstrate increased circulating levels of plasma renin activity (PRA) and aldosterone (65). Furthermore, the administration of losartan, an angiotensin II receptor antagonist, and spironolactone, an aldosterone antagonist, reduces sodium retention (66,67,68). Consequently, the RAAS is thought to be a key mediator of increased renal tubular sodium reabsorption in this setting (65). Angiotensin II is known to enhance sodium reabsorption by acting directly on the proximal convoluted tubule, whilst aldosterone acts primarily on the collecting duct to upregulate epithelial sodium transport proteins (69,70,71). Moderate SNS activity that may be insufficient to alter renal haemodynamics similarly increases sodium retention throughout the nephron via a direct tubular effect. The SNS is thought to be an important additional stimulus for sodium reabsorption (72,73).

The driving force of the tubular dysfunction in portal hypertension remains unclear (74,75). In compensated disease even when there is evidence of splanchnic arterial vasodilatation, a large proportion of patients demonstrate similar serum levels of

renin, aldosterone and nor-adrenaline to both healthy individuals and compensated cirrhotics (10,76). It has therefore been suggested that the underlying pathogenesis in these patients relates to an additional, extremely sensitive sodium retaining mechanism (14). Alternative explanations include the intra-renal generation of angiotensinogen, angiotensin I and angiotensin II as a consequence of subtle local haemodynamic change, or increased tubular sensitivity to the known mediators of sodium retention (77). Recently, it has been shown that in sodium retaining cirrhotic rats there is diminished abundance of 11beta-hydroxysteroid dehydrogenase, which protects the mineralocorticoid receptor from stimulation by circulating glucocorticoids (78).

- Impaired free water clearance

Impaired free water clearance occurs chronologically after sodium retention, being a feature of decompensated cirrhosis, and manifests clinically as dilutional hyponatraemia despite the increased total body sodium (4). Fifty percent of patients with ascites demonstrate hyponatraemia and the prevalence is greater in those with refractory ascites (79). The development of hyponatraemia is now recognised as a strong indicator of poor prognosis in advanced CLD (80).

Vasopressin or antidiuretic hormone (ADH) is considered the chief mediator of impaired free water clearance (4). Vasopressin stimulates aquaporin-2 channel insertion into the epithelial cell membrane increasing the permeability of the distal nephron to water (81). Urinary aquaporin-2 excretion is markedly increased in

patients with cirrhosis, rises in parallel with Child-Pugh class, and demonstrates a significant negative correlation with spontaneous free water clearance (81). Moreover, vasopressin 2 receptor antagonists have been shown to have an aquaretic effect and to increase serum sodium in cirrhotic patients with hyponatraemia and ascites (82,83). A second postulated mechanism of impaired free water clearance is reduced delivery of filtrate to the distal nephron. Greater proximal tubular sodium reabsorption and reduced GFR may therefore be relevant (4).

- Renal haemodynamic dysfunction

Renal haemodynamic dysfunction is a hallmark of advanced portal hypertension-related renal dysfunction (4). With advancing circulatory derangement and neuro-humoral activation there is progressive renal vasoconstriction and a fall in total renal blood flow. Initially, intra-renal compensatory mechanisms maintain the GFR at normal or even elevated levels (84,85,86). Local vasodilators including the prostaglandins and nitric oxide antagonise the vascular effects of the RAAS, the SNS, vasopressin and ET-1 (87,88,89). The importance of the prostaglandins in preserving renal function in decompensated cirrhotics is highlighted by the marked decrease in GFR precipitated by non steroidal anti-inflammatory drugs (87).

Increased post glomerular arteriolar resistance, by maintaining the glomerular hydrostatic pressure, also appears to be a critical factor in sustaining the GFR. Non-azotemic patients frequently demonstrate an increased filtration fraction in the face of a fall in total renal blood flow (85,90). Moreover, a reduced GFR is accompanied

by a reduction in filtration fraction when the total renal blood flow is comparable (90). Angiotensin II, vasopressin and atrial natriuretic peptide are potential mediators of increased efferent arteriole constriction and may serve to counteract the exaggerated afferent vasoconstriction (65,90,91,92,93,94).

In addition to the extra-glomerular renal vascular changes described, it is likely that the neuro-humoral overactivity also has implications for the filtration barrier itself. The contractile mesangial cells that abut the glomerular capillaries are under hormonal regulation and are essential for the physiological control of GFR (95,96,97). In vitro studies have shown that angiotensin II, noradrenaline, vasopressin and ET-1 stimulate mesangial cell contraction and, in contrast, atrial natriuretic peptide and nitric oxide influence cell relaxation (95,98,99,100,101).

With progressive arterial underfilling the accompanying activity of the vasoconstricting systems becomes extreme, and the renal production of vasodilators eventually falls (102). Renal ischaemia stimulates additional intra-renal secretion of ET-1 and increased SNS activity. A self perpetuating cycle develops within the kidney (14,103,104). The balance is tipped in favour of reduced GFR, and hepatorenal syndrome develops.

- 'Hepatorenal syndrome'

Hepatorenal syndrome was first defined in 1996 by The International Ascites Club and updated in 2007 to take into account recent advances in knowledge (Figure 1.2) (4,105). Central to the diagnostic criteria is the exclusion of alternative causes of renal dysfunction such as nephrotoxic drugs, diuretic induced hypovolaemia and renal parenchymal disease. The syndrome is subdivided into two distinct clinical types despite the pathogenetic similarities.

Type 1 hepatorenal syndrome is classically defined as a doubling of the initial serum creatinine concentration to a level greater than 226 $\mu\text{mol/l}$ (2.5 mg/dl) within a 2 week period (105). Most patients demonstrate oliguria, intense sodium and water retention and dilutional hyponatraemia, and circulatory dysfunction is pronounced (106,107). Median survival time without treatment has been estimated at 1.7 weeks with a probability of survival of 25% at one month (106). Type 2 hepatorenal syndrome is characterised by moderate renal impairment, as indicated by a serum creatinine concentration greater than 133 $\mu\text{mol/l}$ (1.5 mg/dl) (105). Renal impairment is less severe than in type 1 hepatorenal syndrome and progresses slowly, whilst the predominant clinical feature is refractory ascites. Median survival time for patients with type 2 hepatorenal syndrome is 6 months (105,108).

Figure 1.2: International Ascites Club “new” diagnostic criteria for hepatorenal syndrome, 2007 (105).

- Cirrhosis with ascites.
- Serum creatinine $>133 \mu\text{mol/l}$ (1.5 mg/dl).
- No improvement of serum creatinine (decrease to level $\leq 133 \mu\text{mol/l}$) after at least 2 days with diuretic withdrawal and volume expansion with albumin. The recommended dose of albumin is 1 g/kg of body weight per day up to a maximum of 100 g/day.
- Absence of shock.
- No current or recent treatment with nephrotoxic drugs.
- Absence of parenchymal kidney disease as indicated by proteinuria $>500 \text{ mg/day}$, microhaematuria (>50 red blood cells per high power field) and/or abnormal renal ultrasonography.

Although the diagnostic criteria for hepatorenal syndrome are widely employed, their dependence on a rigid cut-off value of serum creatinine has rendered them controversial (389,456). Creatinine production is proportional to muscle mass, and is greater in men than women, in younger than older individuals and in black people than in Caucasians despite similar GFR (282). In cirrhosis specifically reduced creatine production by the liver, muscle wasting and increased renal tubular secretion contribute to a falsely low serum creatinine (412,413). Furthermore, the poor prognosis of patients once the hepatorenal syndrome criteria are met is consistent with extreme renal dysfunction that is rarely reversible even with optimal treatment. Earlier identification of renal impairment and intervention is likely to be beneficial.

The Kidney Disease Improving Global Outcomes (KDIGO) group have advised that AKI is defined as an increase in serum creatinine $\geq 26.4 \mu\text{mol/l}$ (0.3 mg/dl) in 48 hours, or a rise to ≥ 1.5 times baseline within 7 days (457). This classification has been shown to predict survival also in patients with cirrhosis (458). Consequently, a recent working party has proposed new diagnostic criteria for hepatorenal syndrome based on the KDIGO and NKF KDOQI (National Kidney Foundation Kidney Diseases Outcomes Quality Initiative) guidelines for defining AKI and CKD, respectively (Figure 1.3) (389). Yet, opinion remains divided amongst Hepatologists and the International Ascites Club has advocated further research before adopting this proposal (456).

Figure 1.3: Working party proposed diagnostic criteria for kidney dysfunction in cirrhosis, 2011 (389).

AKI	Rise in serum creatinine of $\geq 50\%$ from baseline or a rise of serum creatinine by $\geq 26.4 \mu\text{mol/l}$ ($\geq 0.3 \text{ mg/dl}$) in < 48 hours. Hepatorenal syndrome type 1 is a specific form of acute kidney injury.
CKD	GFR $< 60 \text{ ml/min}$ for > 3 months calculated using MDRD6 formula. Hepatorenal syndrome type 2 is a specific form of CKD.
Acute-on-CKD	Rise in serum creatinine of $\geq 50\%$ from baseline or a rise of serum creatinine by $\geq 26.5 \mu\text{mol/l}$ ($\geq 0.3 \text{ mg/dl}$) in < 48 hours in a patient with cirrhosis whose GFR is $< 60 \text{ ml/min}$ for > 3 months calculated using MDRD6 formula.

Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; GFR, glomerular filtration rate.

Why some patients develop type 1 hepatorenal syndrome and others type 2 has not yet been ascertained. In 50% of those with type 1 the onset is chronologically related to a precipitating event such as severe bacterial infection, gastrointestinal bleeding or large volume paracentesis (106). Mean arterial pressure is significantly lower and there is greater stimulation of the RAAS and SNS when patients are compared with those classified as type 2, suggesting more marked splanchnic arterial vasodilatation (109).

Impaired cardiac function may also play a role (Figure 1.1). Patients who later develop hepatorenal syndrome have a lower cardiac output, albeit within the normal range (109). Moreover, the onset of hepatorenal syndrome is accompanied by a further significant reduction in cardiac output in those with type 1, but not type 2 (109,110). A possible explanation for the fall in cardiac output is the altered vascular compliance and hence cardiac preload. However, a cirrhotic cardiomyopathy has been described that consists of myocardial hypertrophy and diastolic dysfunction, with an abnormal inotropic and chronotropic response to exercise (111,112). Altered sensitivity to sympathetic activity with down regulation of β adrenergic receptors of the cardiomyocyte plasma membrane has been described in animal models (113,114).

It is therefore postulated that type 1 hepatorenal syndrome occurs as a result of an acute worsening of arterial vasodilatation on the background of advanced circulatory dysfunction, exacerbated by a relatively low cardiac output state. Intense activity of systemic and intra-renal vasoactive mediators leads to a downward spiral of renal

hypoperfusion, ischaemia, and impaired GFR. In contrast, type 2 hepatorenal syndrome probably represents the gradual decline in renal haemodynamics associated with advancing disease, with intra-renal compensatory mechanisms coming into play (14,109).

The role of the systemic inflammatory response in portal hypertension-related renal dysfunction

The systemic inflammatory response is increasingly recognised as a pathogenetic factor in the circulatory dysfunction of advanced cirrhosis, in the absence of overt infection (Figure 1.1). Patients with Child-Pugh Class C cirrhosis demonstrate an increased frequency of bacterial translocation of enteric organisms to mesenteric lymph nodes (115). Bacterial DNA is present in the blood of approximately 40% of non-infected patients with ascites (116,117,118). In addition, the plasma levels of lipopolysaccharide binding protein (LBP), which is considered a better marker of transient endotoxaemia given its longer half-life, are also elevated (119).

In rats with cirrhosis bacterial translocation is associated with eNOS derived nitric oxide overproduction in the mesenteric vasculature, which appears to aggravate arterial vasodilatation (120). Humans with advanced cirrhosis display increased mesenteric lymph node tumour necrosis factor- α (TNF- α) expression (121). Ascitic patients with high LBP levels have higher circulating levels of TNF- α , interleukin (IL) -6, soluble TNF and lipopolysaccharide receptors, and nitric oxide metabolites, and a more pronounced circulatory dysfunction (119). Moreover, they

demonstrate a greater increase in circulating monocytes that have an enhanced capacity for TNF- α expression, which correlates directly with blood TNF- α levels (122). The exaggerated immuno-haemodynamic derangement is reversed by the administration of norfloxacin, an effect that is not reproduced in cirrhotics with ascites and normal LBP levels (119,122).

Tying this together, bacterial translocation and the secondary systemic response may result in increased nitric oxide generation and exacerbate the pre-existing portal hypertensive syndrome. Indeed, long-term prophylactic antibiotics in patients with advanced CLD reduce the incidence of hepatorenal syndrome and improve survival, independent of the prevention of infection (123).

Treatment of portal hypertension-related renal dysfunction

Despite progress in our understanding of the mechanisms underlying the spectrum of portal hypertension-related renal dysfunction treatment options remain limited. In the earlier phase, management is supportive aiming to minimise patient morbidity as a result of sodium and water retention. In later phases specific therapies are employed to increase the effective circulating volume, renal perfusion and GFR, both directly and via a reduction in endogenous vasoactive mediators.

The onset of type 1 hepatorenal syndrome should prompt a search for the precipitating event. In particular, infection should be considered and there should be a low threshold for antibiotics even in culture negative patients. Intravascular volume

status should be assessed and corrected as appropriate. Type 2 hepatorenal syndrome signifies end stage disease and therapy primarily focuses on management of refractory ascites and hyponatraemia. Non steroidal anti-inflammatory drugs and RAAS antagonists are avoided because of the reno-protective effect of the prostaglandins and angiotensin II, respectively.

- Management of sodium and water retention

In patients with clinically apparent ascites a salt restricted diet (80 to 120 mmol sodium per day) is recommended and escalating doses of diuretics are employed (124). Oral fluid restriction is poorly adhered to and seldom effective (124).

Large volume paracentesis is first line therapy for patients with grade 3 ascites prior to initiation of maintenance diuretics, or may be the main stay of treatment in refractory ascites (124). AKI is not a contraindication to paracentesis. In this setting, large volume paracentesis with albumin administration is accompanied by an increase in renal perfusion pressure, creatinine clearance and fractional excretion of sodium possibly as a result of the fall in intra-abdominal pressure (125).

A meta-analysis has suggested that vasopressin 2 receptor antagonists reduce the time to first paracentesis, and increase the serum sodium. However, the authors concluded that the cost and modest benefit did not justify their routine clinical use (126).

- Therapies that increase the effective circulating volume and renal function

Terlipressin is a vasopressin analogue with predominant vasopressin 1, although some vasopressin 2 receptor effects (127). In patients with ascites without hepatorenal syndrome terlipressin resulted in improved systemic haemodynamics, a fall in circulating neurohumoral mediators, reduced renal arterial resistive index, improved GFR and increased urinary sodium excretion (128,129). When ascites is non refractory the increase in GFR occurs in association with an increase in filtration fraction but no change in total renal blood flow, supporting the concept that some of the benefit may be mediated by post glomerular vasoconstriction (129).

Randomised controlled trials have demonstrated that terlipressin reverses type 1 hepatorenal syndrome and results in a small reduction in short term mortality (130). Nevertheless, prognosis remains poor with a reported 6-month transplant free survival of only 13% (131,132). In type 2 hepatorenal syndrome renal failure invariably recurs following treatment withdrawal (105). However, terlipressin may be beneficial as a bridge to liver transplantation in this cohort (133). Noradrenaline appears to be as effective as terlipressin for treatment of type 1 hepatorenal syndrome, but has practical disadvantages and is generally used only in countries where terlipressin is not available (134,135,136).

Intravenous human albumin has multiple beneficial properties in patients with portal hypertension. In addition to the volume loading provided by less concentrated

preparations and increase in oncotic pressure, albumin binds endotoxin, has anti-inflammatory and antioxidant effects, and consequently may impact on endothelial and cardiac dysfunction (137). Administration of albumin results in an increase in systemic vascular resistance and improvement in cardiac function, which is not seen following hydroxethyl starch (138,139).

In patients with ascites receiving diuretics, intravenous albumin therapy is associated with a faster rate of ascites mobilisation and a lower probability of ascites reaccumulation (140). Furthermore, albumin has been shown to prevent the systemic and renal dysfunction precipitated by large volume paracentesis and spontaneous bacterial peritonitis (138,141,142,143). In those with hepatorenal syndrome, albumin and terlipressin resulted in reversal of renal dysfunction in 77%, compared with 25% of patients receiving terlipressin alone (144).

- Non pharmacological therapies

Transjugular intra-hepatic portosystemic shunt (TIPSS) has a role in the management of select patients with portal hypertension-related renal dysfunction. Although the immediate effect is an exacerbation of the hyperdynamic state as evidenced by an increase in cardiac output and reduction in systemic vascular resistance, longer term the fall in portal pressure is associated with improved circulatory and neuro-humoral derangement (145,146). Patients demonstrate a marked and sustained fall in PRA, and plasma aldosterone and noradrenaline concentrations (147,148,149,150). In those with refractory ascites, and in hepatorenal syndrome, TIPSS results in

increased total renal blood flow and GFR, and less sodium retention (147,148,149,150).

Meta-analyses of randomised controlled trials confirm that TIPSS in refractory ascites is associated with a reduced recurrence of ascites when compared with large volume paracentesis (151,152). In this group there may be a small but statistically significant positive effect on survival (153). Nevertheless, patients with poor synthetic function are unlikely to benefit and a serum bilirubin greater than 85 $\mu\text{mol/l}$ is considered an absolute contra-indication (124,153). The applicability of TIPSS once hepatorenal syndrome develops is likely to be limited given the high prevalence of jaundice and encephalopathy (124).

Haemofiltration can be useful in patients with hepatorenal syndrome when there is a reversible super-imposed component such as infection exacerbating the haemodynamic and renal dysfunction. However, in patients with end stage disease and type 2 hepatorenal syndrome renal replacement therapy (RRT) has no role. Albumin dialysis with the molecular adsorbent recirculating system (MARS) has been shown in a single small randomised controlled trial to improve renal function in type 1 hepatorenal syndrome when compared with haemodiafiltration alone (154). Yet, whether the effects are sustained is unknown and MARS remains an experimental therapy only at present (124).

Liver transplantation is the definitive treatment for portal hypertension-related renal dysfunction offering a clear survival advantage (155). Most patients with hepatorenal

syndrome demonstrate an improvement in renal function, although post transplant CKD is more common in this group (155). Duration of reduced GFR is important in influencing outcomes, probably reflecting the development of secondary acute tubular necrosis (156,157). Combined liver kidney transplantation is recommended for all patients with stage 3 AKI or a GFR of less than 25-35 ml/min for more than 4 weeks (158). This population is considered to gain survival benefit from combined liver kidney transplantation over liver transplantation alone, although the supporting literature is flawed by the heterogeneity of the cohorts studied (158).

1.3 Acute liver failure: Acute kidney Injury

ALF is a condition characterised by sudden severe liver injury that occurs in the absence of pre-existing liver disease (159). In contrast to cirrhosis ALF is relatively rare but usually affects younger patients, and has a high mortality rate and major resource implications (160).

The poor prognosis of ALF not only reflects an imbalance between hepatocyte death and regeneration, but also the development of secondary extra-hepatic complications (161). In a recent series 30% of listed patients not surviving to liver transplantation had intra-cranial hypertension and 95% had multi-organ failure (162). While some advances have been made in our understanding of and treatment options for hepatic encephalopathy in this setting, renal dysfunction has received little scientific interest. Renal failure is common. More than 40% of patients with severe encephalopathy have been reported to demonstrate a serum creatinine rise to greater than 400 $\mu\text{mol/l}$ (163). Furthermore, renal failure is associated with increased mortality, emphasised by the inclusion of serum creatinine in the Kings College Hospital (KCH) prognostic criteria (164,165). It is well recognised that AKI has implications for distant organ function in other disease processes. Via immunomodulation AKI itself may drive cardiac, lung, and brain, as well as liver injury (166,167,168). Therefore, it follows that the minimisation of renal dysfunction in patients with ALF could alter the outcome of patients not suitable for liver transplantation, and in those listed could impact on survival to transplantation and post transplant outcomes.

The pathophysiology of the haemodynamic and renal derangement that accompanies ALF remains unclear. Some authors postulate that the hyperdynamic state mirrors the circulatory dysfunction of cirrhosis with portal hypertension (105,169,170,171,172). However, ALF has distinct clinical features and the pathogenesis, particularly in hyperacute liver failure, may be more in keeping with sepsis (173,174). Both infected and non infected patients with ALF have high circulating levels of cytokines and demonstrate the systemic inflammatory response syndrome (SIRS) (175,176). Moreover, SIRS is linked with progression of hepatic encephalopathy and mortality (175,176,177). Interestingly, in early unpublished work when liver biopsies of patients with and without renal failure were compared no difference in the proportion of surviving hepatocytes could be demonstrated (178). Significant inter-individual differences in the systemic inflammatory response to infection and other forms of injury have been observed, which may be genetically predetermined (179,180). Thus, it seems possible that AKI in patients with ALF is not solely governed by the degree of liver injury but also by the systemic inflammatory response.

The systemic circulation in acute liver failure

In ALF, patients consistently demonstrate a low systemic vascular resistance and high cardiac output state (170,171,172,181). However, the regional haemodynamic changes have been poorly reported and the specific vascular beds to undergo dilatation remain unknown. Is there widespread vasodilatation as occurs in sepsis or

localised splanchnic vasodilatation with cerebral, femoral, brachial and renal vasoconstriction as demonstrated by patients with cirrhosis (11,12,182,183)?

- Hepatic blood flow

Rats with ALF have a marked increase in total hepatic blood flow, and an increase in the hepatic arterial to portal venous flow and oxygen delivery ratios (184). The increase in hepatic blood flow is not determined by cardiac output, consistent with local regulation. This replicates observations in animal models of sepsis (184,185,186). In humans reported data is also suggestive of an increase in total hepatic blood flow (181,187). Contrary to findings in the rat model, hepatic arterial resistance index measured using ultrasound Doppler is elevated and may reflect the severity of liver failure (186,188).

Portal hypertension is a feature in most, but not all patients. The hepatic venous pressure gradient is elevated correlating with the degree of reticulin collapse, and there is an increase in the longitudinal diameter of the spleen (172,188). Those with ascites have a higher hepatic venous pressure gradient than those without. In fact, 80% of patients with ascites have a hepatic venous pressure gradient greater than 12 mmHg, compared with only 30% of patients with no ascites (172). Moreover, portal hypertension is present in the majority of patients classified as subfulminant liver failure and infrequent in more acute disease (172).

- Cerebral blood flow

Reported cerebral blood flow values are highly variable reflecting confounding factors and timing of measurements (189,190,191,192). Studies assessing the response of cerebral blood flow to altered partial pressure of carbon dioxide (CO₂) and systemic arterial pressure are consistent with impaired autoregulation, favouring a relatively vasodilated state (193,194,195). Intra-cranial pressure is critically determined by cerebral blood flow (192,196,197).

- Peripheral blood flow

Peripheral vasodilatation is generally assumed to be present by experienced intensivists and hepatologists (169). A surgical model of ALF in pigs has suggested reduced hind leg vascular resistance (198,199). However, in an uncontrolled study of patients lower extremity blood flow was said to be similar to previously reported values for healthy volunteers, although no adjustment was made for systemic haemodynamics (200).

- Renal blood flow

As in sepsis, but in contrast to hepatorenal syndrome, renal blood flow measurements in animal models have yielded conflicting results (183). In one study, renal vascular resistance was increased and renal blood flow reduced compared to controls (201). Renal blood flow correlated with portal pressure and intra-hepatic porto-systemic

shunting but not arterial blood pressure (202). On the other hand, in another model a relative reduction in renal vascular resistance and no change in renal blood flow compared to shams were demonstrated (199). Authors attributed these apparent contradictory findings to different stages of the disease, postulating that generalised vasodilatation occurs early and renal vasoconstriction late (199). Circulatory resuscitation and the animal model used are additional likely influential factors. Human studies from the 1970s and 80s demonstrated reduced renal plasma flow and GFR in the presence of severe hepatic encephalopathy (171,203).

The spectrum of renal dysfunction in acute liver failure

The spectrum of renal dysfunction in ALF has not been well described. Wilkinson et al observed that 50% of patients with ALF of mixed aetiology and severe encephalopathy without renal failure had sodium retention and impaired free water clearance (203). This group had a slightly reduced renal plasma flow and GFR compared to patients with normal renal tubular function. Transudative ascites occurred in one case series in 75% of patients with subfulminant liver failure, and only 33% with fulminant liver failure (172). Exudative ascites has also been described in viral hepatitis and occurs in the absence of portal hypertension (172). Anecdotally, ascites is not seen in hyperacute liver failure such as following paracetamol overdose. Once renal failure has developed 30-65% of patients have been reported to have urinary features of acute tubular necrosis, and this has been observed chronologically after functional renal impairment (164,204).

Additional factors that may contribute to the development of renal dysfunction in ALF include dehydration secondary to vomiting, infection, disseminated intravascular coagulation and nephrotoxic drugs (178,205,206,207,208). Paracetamol has been shown to induce renal tubular cell necrosis and apoptosis in vitro and there are case reports of isolated renal failure following paracetamol overdose in the absence of hepatic injury (209,210,211).

Data is currently lacking to confirm the spectrum of renal dysfunction in ALF. However, clinical observations and Navasa's portal hypertension study suggest that the slower onset forms of ALF may have haemodynamic and renal changes with parallels to the circulatory changes of cirrhosis and portal hypertension (172). However, in fulminant liver failure differing mechanisms may be key.

The systemic inflammatory response to acute liver failure

SIRS is the clinical sequelae of a massive inflammatory cascade that results from systemic cytokine release (212). The illness that characterises SIRS is considered a continuum of clinical and pathophysiological severity with multi-organ dysfunction at the extreme end (212). SIRS is most commonly associated with infection, when it is termed sepsis. However, SIRS is also present in a variety of non-infectious disease processes such as acute pancreatitis, trauma, haemorrhagic shock, and burns (212). In ALF patients frequently demonstrate SIRS even in the absence of clinical sepsis (175,176). Multi-organ dysfunction is present in the majority of listed patients who do not survive to transplantation, and SIRS has been linked with organ dysfunction

and outcome (162,175,176,177). It therefore seems probable that SIRS plays a role in the haemodynamic and renal complications of ALF.

- Current hypothesis for the inflammatory response to injury

It is postulated that the inflammatory response to injury is dictated by the balance of pro- and anti-inflammatory mediators (213,214,215). At the initial site of injury or infection macrophages, polymorphonuclear phagocytes, endothelial cells and complement are activated with the release of pro-inflammatory mediators to destroy damaged tissue and antigens, and to promote wound healing. Anti-inflammatory agents attempt to down regulate this effect and prevent further tissue damage.

If the primary event is sufficiently severe the inflammatory response may spill out into the systemic circulation to allow recruitment of neutrophils, T and B cells, platelets and coagulation factors and, hence, limit the local injury. The inflammatory and compensatory anti-inflammatory systemic responses occur simultaneously and maintain homeostasis, though one process may be favoured over another. When pro- and anti-inflammatory responses go unchecked SIRS and the compensatory anti-inflammatory response syndrome (CARS) may manifest, respectively. CARS is characterised by relative immunosuppression and an increased susceptibility to infection. The balance between SIRS and CARS determines outcome. Persistent imbalance, termed immunological dissonance, may result in death from overwhelming inflammation, or from failure to clear infection and to promote organ recovery.

- The systemic inflammatory response in sepsis and other multiple organ dysfunction syndromes

In sepsis, bacterial products trigger the systemic inflammatory response via pattern recognition receptors (PRRs) on innate immune cells (460,461). It is this apparently critical initial step that promotes the release of inflammatory mediators, including cytokines such as IL-1, IL-6, TNF- α , IL-12, interferons, chemokines, adhesion molecules, growth factors, tissue-degrading enzymes such as metalloproteinases, and enzymes that generate cyclooxygenase-2 and iNOS (466). Examples of PRRs include Toll-like receptor (TLR)4 that detects lipopolysaccharide, and TLR2 that detects multiple microbial products from bacteria, fungi and viruses (461). The importance of the TLRs in sepsis is demonstrated by TLR deficient animal models, and genetic polymorphisms in humans have been linked with an augmented susceptibility to infection (461).

The systemic pro-inflammatory and anti-inflammatory response follows simultaneously, although in early sepsis the former predominates (462). The severity of the response is determined by multiple pathogen and host factors (462). Some patients will experience an overwhelming rapidly fatal pro-inflammatory cascade, as seen in meningococcal septicaemia and toxic shock syndrome (462,463). The majority, however, progress to a protracted anti-inflammatory or immunoparesis phase when there may be failure to clear the infection, development of secondary infections and a high risk of death (463). At autopsy a continued septic focus is almost universally present (464). Interestingly, despite apoptosis and necrosis being

detected in most organs, the extent of cell death is not considered enough to explain organ failure (465). It is speculated therefore that cell “hibernation” contributes to organ dysfunction in these patients.

A similar syndrome occurs in patients with tissue injury without sepsis, such as in acute pancreatitis, trauma, major haemorrhage and burns (212). Here, endogenous products of cell injury called damage-associated molecular patterns (DAMPs) are the TLR ligands (461,466,467). DAMPs include nucleic acids, histones, uric acid crystals, adenosine triphosphate, cytochrome C, S100 molecules, and high-mobility group box-1 protein (HMGB1) (461). HMGB1 has received particular attention, and anti-HMGB1 neutralising antibody has been shown to attenuate organ damage in experimental models of acute pancreatitis, haemorrhage, trauma and hepatic ischaemia-reperfusion injury (468,469,470,471). The downstream events after DAMP-TLR interaction are not identical to those initiated by pathogens in sepsis (466). Nevertheless, the clinical picture of an initial primarily pro-inflammatory multiorgan dysfunction followed by delayed immunosuppression is comparable (472).

It should be mentioned that endotoxaemia is frequently observed in sepsis and other inflammatory conditions, and correlates with the development of multiorgan failure (473,474,475,476). The gut is especially vulnerable to ischaemia-reperfusion injury during haemodynamic instability. The “gut hypothesis” suggests that disruption of the mucosal barrier resulting in bacterial translocation, and the release of

nonbacterial gut-derived factors, plays a key role in distant organ injury and dysfunction in multiple organ dysfunction syndrome (477).

- Evidence for the systemic inflammatory response in acute liver failure

Patients with ALF demonstrate increased levels of circulating pro- (TNF- α , IL-1, IL-6 and IL-8) and anti-inflammatory cytokines (IL-10) (216,217,218,219,220,221,222,223). Moreover, systemic levels of the acute phase protein c-reactive protein are increased, and α 1 anti-trypsin and fibrinogen levels are greater than expected (216,218,223). However, cytokine concentrations are highly variable and correlation with mortality is less consistent (216,220,221,222,223). This disparity may reflect diverse study populations or methodological difficulties (216,220,221,223,224). Importantly, immunoassays detect only free, circulating inflammatory mediators and not those bound to cells receptors (179). In addition, cytokine release has circadian periodicity and the inflammatory response is dynamic and of variable duration: in the majority of studies inflammatory mediators were measured at a single time point (179).

Sheron et al performed serial measurements of IL-6 and TNF in a group comprised mainly of paracetamol-induced liver failure and commented that no underlying trend was demonstrated over time (221). Frequency of measurements was not documented and data not shown. Nagaki et al demonstrated serial TNF- α , IL-6 and IL-10 levels in a small and variable number of patients with predominantly viral-induced liver injury treated with plasma exchange (220). Again no relationship was seen.

TNF- α has two specific receptors that may be shed following binding and circulate as soluble ligand-receptor complexes (225). Soluble TNF receptors I (p55) and II (p75) have a far longer half-life than free TNF- α and, consequently, may be more sensitive indicators of the inflammatory response (226). In ALF soluble TNF receptor I and II levels are increased compared to healthy controls, and elevated soluble TNF receptor I levels correlate with non survival (217,219,220). The soluble TNF receptors, by competing with cell surface receptors, may play an anti-inflammatory role or, alternatively, may act as a 'slow release reservoir' and augment the effects of TNF- α (227). Keane et al observed reduced TNF- α neutralization capacity of plasma from patients with ALF in vitro despite increased levels of soluble TNF receptors (217).

The ratio of pro- to anti-inflammatory mediators may be more relevant than absolute values. Sekiyama et al found that the ratio of IL-1 receptor antagonist to IL-1 β at time of hospitalisation was threefold lower in non survivors than in survivors (216).

- Evidence for immune cell dysfunction in acute liver failure

A hallmark of CARS is immune cell dysfunction (213,214). In addition to an increase in circulating anti-inflammatory mediators, T-cell anergy and monocyte deactivation has been described in sepsis, burns and trauma (214,224,228). T-cell, B-cell and dendritic cell apoptosis is seen in sepsis and acute pancreatitis, which induces further anergy and anti-inflammatory cytokine release (214,229).

In ALF research has focussed on monocyte dysfunction. Circulating monocytes in vitro from patients with ALF demonstrate similar spontaneous but reduced lipopolysaccharide stimulated IL-6 production when compared with controls (218). TNF production has been reported to be normal or increased depending on aetiology and almost certainly timing (218,230,231). However, in paracetamol-induced liver failure both reduced spontaneous and stimulated production of TNF is associated with poor outcome (218,230). An expansion of IL-10 producing monocytes and an increase in IL-10 secretion following endotoxin stimulation supports the concept of a shift toward anti-inflammatory monocyte activity (215,232,233). The HLA-DR molecule is a key antigen-presenting surface molecule and reduced HLA-DR expression by monocytes is a feature of sepsis, predicting poor outcome (234). Similarly, ALF patients demonstrate a decrease in HLA-DR expression that is more marked in non-survivors (224).

Neutrophil dysfunction consisting of reduced production of superoxide and hydrogen peroxide, and impaired phagocytosis has been described (235,236). The administration of granulocyte colony-stimulating factor (G-CSF) in vitro and in vivo, which is known to induce neutrophil proliferation, maturation and increase superoxide production, improved neutrophil phagocytosis and killing (236,237). Endogenous G-CSF levels demonstrate no association with mortality (238).

- Source of the systemic inflammatory response to acute liver failure

The trigger for the systemic inflammatory response to ALF remains unclear. However, the observation that hepatectomy results in haemodynamic stabilisation and reduced intracranial pressure is in agreement with the concept of the liver as the driving force (478). ‘Spill over’ of inflammatory mediators from the necrotic liver may occur, or necrotic cells may enter the circulation and cause immune stimulation (213,214).

Echoing other non-infectious inflammatory conditions, there is a growing body of evidence to support a critical role for DAMPs. Levels of HMGB1 are increased in the circulation of animals and humans with acute liver injury (479,480,481,482). Moreover, anti-HMGB1 has been shown to attenuate liver damage, reduce plasma cytokines levels and improve survival in D galactosamine induced-ALF in rats (479). Of note, although HMGB1 is initially passively secreted by cells undergoing necrosis, it is also later released as an inflammatory mediator by monocytes and macrophages in a hyper-acetylated form (482). Circulating acetylated HMGB1 levels, representing activation of the immune response, have been found to be elevated in patients who died or were transplanted, but not in spontaneous survivors of ALF (482). Other products of cell death that have been demonstrated in the systemic circulation in ALF include DNA fragments, mitochondrial products, keratin-18 and nucleosomes (480,481,483).

Several DAMP receptors that are known to be involved in innate immune cell activation have been examined as potential stimuli of the systemic inflammatory response to ALF. TLR4 expression is increased in the liver and serum monocytes in experimental models, and TLR4 knockout/antagonism is accompanied by less liver injury, a delayed onset of hepatic encephalopathy, less lung and renal injury, and superior survival (484,485,486,487). Similarly, blockage of TLR9 and formyl peptide receptor 1 (FPR1) results in an attenuated systemic inflammatory response, and protects against liver and distant organ damage (489). In humans with acute liver injury, the PRR pentraxin 3 is elevated in plasma, and the liver has been confirmed as a potential source (488). Furthermore, pentraxin 3 levels correlate with circulating cytokine levels, organ dysfunction including brain and kidney, and outcome (488).

The “gut hypothesis” is also likely applicable. Endotoxaemia certainly is common, perhaps in part reflecting impaired hepatic clearance, and has been correlated with worse survival (205,490). Inhibition of endotoxin in a surgical model of ALF blunted the rise in circulating TNF and IL-6 (239).

Finally, impaired metabolism of cytokines is probably important. Inadequate hepatic metabolism of inflammatory mediators has been suggested as a factor in patients with hepatic insufficiency (179). The kidneys remove both pro- and anti-inflammatory cytokines possibly explaining some of the association between renal dysfunction and death (240).

- Genetic predisposition to the systemic inflammatory response to acute liver failure

There are significant inter-individual differences in the systemic inflammatory response to infection and other forms of injury, which may be genetically predetermined (179,180). The TNF genotype B1B1 has been negatively associated with the development of severe encephalopathy in paracetamol-induced liver failure (241). Large scale studies are required to examine the relationship between genetic polymorphisms and outcome measures.

The role of the systemic response to acute liver failure in the aetio-pathogenesis of acute kidney injury

Given the overwhelming evidence of a systemic inflammatory response to ALF it seems probable that this plays an important and potentially critical role in the pathogenesis of the circulatory and renal dysfunction in these patients.

In sepsis, the haemodynamic derangement is primarily attributed to endothelial dysfunction (213). Inflammatory cytokines induce endothelial cell activation with a loss of vascular integrity and a shift towards a pro-thrombotic, pro-inflammatory state. Secondary upregulation of nitric oxide via iNOS, prostaglandins via COX-2, and endothelins occurs mainly in the underlying smooth muscle. The pattern of these vasoactive mediators determines vascular response and, thus, organ dysfunction (242). In ALF, serum IL-6 levels correlate with low mean arterial pressure, low

systemic vascular resistance and oxygen consumption (221). Nitric oxide and ET-1 and -3 levels are elevated (243,244). Attenuation of the systemic inflammatory response to ALF in rats prevented the fall in mean arterial pressure (239). Moreover, the therapeutic application of hypothermia in patients is accompanied by an improvement in systemic haemodynamic measurements (197,245).

The pathogenesis of AKI in sepsis involves both haemodynamic and non haemodynamic factors (183). Firstly, there is a reduced glomerular filtration pressure resulting in a fall in GFR. Whether this reflects reduced renal blood flow or intrarenal haemodynamic alterations remains unknown. It has been postulated that there may be relative efferent arteriole vasodilatation with preserved or even increased total renal blood flow (183,206). Secondly, there is evidence of direct renal tubular cell death. Post-mortem kidney biopsies from patients who died of septic shock demonstrate as well as acute tubular necrosis an intense leukocyte infiltration of glomeruli and interstitial capillaries, and the presence of tubular cell apoptosis (246). Endotoxin, either directly or via systemic cytokine release, may induce renal tubule and glomerular endothelial apoptosis in experimental models (247). TNF- α especially appears to be an important player in the renal injury of sepsis (183,206,247). Supporting the hypothesis that AKI in ALF shares similar mechanisms the only study to examine circulating inflammatory mediators and their association with renal dysfunction found a positive correlation between plasma TNF, and soluble TNF receptors, and serum creatinine (221).

Management of acute kidney injury in acute liver failure

The management of AKI in ALF is supportive. RRT is often instituted early and in the absence of oliguria based on the premise that the removal of inflammatory and other mediators may act to stabilise circulatory dysfunction and minimise organ injury (169,247). Continuous venovenous haemodiafiltration is the method of choice to avoid haemodynamic instability and rapid electrolyte and fluid shifts, which may increase the risk of cerebral oedema (169). Controlled hypothermia may also be beneficial (197). In contrast to cirrhotic liver disease, terlipressin worsens cerebral hyperaemia and intracranial hypertension and should be avoided (248).

1.4 Renal dysfunction after liver transplantation

Liver transplantation is the definitive treatment for portal hypertension-related renal dysfunction in cirrhosis, and AKI in ALF. Yet, liver transplantation itself is complicated by renal disease. During the peri-operative period approximately one third of all liver transplant recipients develop AKI, and one quarter require RRT (249,250). Thereafter, chronic kidney dysfunction is common with a 5-year cumulative incidence of stage 4/5 CKD as high as 18% (8). AKI and CKD are major causes of morbidity and mortality in liver transplant recipients.

Peri-operative acute kidney injury

AKI immediately after liver transplantation is multi-factorial in origin, although pre-existing renal dysfunction plays an important role. CKD is a consistent risk factor for AKI in other settings, possibly as a result of haemodynamic dysfunction and altered renal autoregulation, and an increased pre-disposition to renal injury (251). Liver transplant patients demonstrate a spectrum of renal dysfunction. In liver transplantation for cirrhosis the overall reported prevalence of ascites and hyponatraemia is 80% and 30%, respectively (252,253). The frequency of intrinsic renal disease is less clear, although many patients have liver disease that may cause co-existent renal injury, and diabetes mellitus and hypertension are common (8). In one series of unselected patients who underwent renal biopsies at the time of transplantation universal glomerular abnormalities were observed (254). Several studies have confirmed that an increased pre transplant serum creatinine is a predictor of post operative AKI (249,255,256).

Intra-operative events also play a key role. The type of transplant surgery is relevant as the piggyback technique, which preserves venous continuity, has been associated with a reduced incidence of acute renal dysfunction (257,258). Intra-operative hypotension and need for inotropes are common predictors of AKI (249,250). The greatest intra-operative haemodynamic derangement typically occurs at the time of graft reperfusion and patients with post reperfusion syndrome, the extreme manifestation, have a marked increase in the frequency of severe renal impairment (259). Similarly, blood transfusion requirements have been related to AKI in many

observational studies possibly reflecting the severity of surgical haemorrhage (250,260). Alternatively, excessive transfusion may be causal and increase blood losses. When a low central venous pressure is maintained during the pre-anhepatic phase via the avoidance of plasma transfusion and the use of intra-operative phlebotomy less renal dysfunction is observed (261).

Post-operatively, intra-abdominal hypertension demonstrates a relationship with kidney dysfunction, possibly as a result of reduced renal blood flow, intra-renal redistribution of blood, or renal congestion (260,262). The administration of a calcineurin inhibitor (CNI) further compromises renal perfusion and function. Tacrolimus and cyclosporine cause acute, dose-dependent, renal vasoconstriction and a fall in GFR (263). Such effects have been attributed to an imbalance between vasoactive substances including endothelin and prostaglandins that are also implicated in the pathogenesis of hepatorenal syndrome (4,263). It has therefore been postulated that the greater haemodynamic and neuro-humoral derangement of cirrhotic patients may result in an increased susceptibility to the nephrotoxic effects (264). Delayed and lower dose peri-operative tacrolimus has been demonstrated to be beneficial for short term post transplant renal function (265).

- Clinical relevance of acute kidney injury after liver transplantation

The well recognised initial clinical consequences of AKI are electrolyte and acid base disturbance, and fluid overload. Liver transplant recipients with AKI have a prolonged intensive care stay and hospitalisation. Moreover, AKI is an independent

risk factor for mortality in Critical Care (266). The direct and indirect financial burden of AKI is significant (267).

Beyond the peri-operative period, AKI has important ramifications for renal function (251). In non transplant populations, such as patients undergoing major vascular surgery, the occurrence of perioperative AKI is associated with an increased risk of CKD (268). Furthermore, patients requiring dialysis for AKI who are dialysis-free at the time of hospital discharge are 3 times more likely to develop end-stage renal failure (269). Similar observations have been made in liver transplant recipients (8). Animal models have confirmed that AKI can cause permanent structural damage, with progressive tubulo-interstitial fibrosis and lasting implications for renal function (270). Increasing severity of AKI is associated with greater long term mortality in a graduated manner (271). Therefore, in a time of increasing graft longevity necessitating a shift of focus to nongraft related complications, AKI is likely a potentially modifiable factor involved in late post transplant morbidity and mortality.

Chronic kidney disease after liver transplantation

The development of CKD after liver transplantation can be predicted by multiple patient factors. Pre-transplant renal function is consistently associated with renal outcome. With increasing severity of renal impairment there is an increased risk of CKD (8). Moreover, as previously discussed, the duration of renal dysfunction is important probably reflecting the onset of secondary acute tubular necrosis in hepatorenal syndrome or intrinsic kidney disease (156,157). The vast majority of

patients requiring RRT prior to transplantation for less than 30 days are removed from dialysis post transplant, compared to only 10% of those requiring it for more than 3 months (272).

Other pre transplant risk factors include older age, female gender, white ethnicity, hepatitis C and diabetes mellitus (8,273). Confirming the multi-factorial nature of CKD after liver transplantation Pilleboute found vascular lesions in 65% of renal biopsies performed a mean of 5 years post surgery, tubulointerstitial lesions attributed to hydroxyethyl starch in 61%, thrombotic microangiopathy likely secondary to interferon alpha therapy for hepatitis C in 50%, CNI toxicity in 46% and diabetic lesions in 34%. Two patients out of 26 were diagnosed with IgA nephropathy. Lesions belonging to multiple categories were present in most cases (274).

On the whole liver transplant recipients demonstrate a steep decline in GFR during the first post operative weeks with relative stabilisation thereafter, and 6 and 12 month post transplant GFR are invariable predictors of chronic renal dysfunction (8,264,275,276,277,278). Such a dramatic loss of renal function is attributed to peri-operative AKI and the CNIs (263,264,279,280). In addition to the acute haemodynamic consequences of cyclosporine and tacrolimus the CNIs cause chronic renal damage with tubulo-interstitial fibrosis, an effect that is considered dose-independent (263). Patients receiving cyclosporine in the non transplant setting for autoimmune uveitis demonstrate an almost identical change in renal function over time (281). Despite no change in renal plasma flow the uveitis cohort had a

progressive decline in GFR with irreversible glomerular and tubulointerstitial lesions (281). Not unsurprisingly, in liver transplant patients the CNI troughs levels do not generally demonstrate a relationship with CKD, although a higher 1 month cyclosporine trough level and a greater daily and cumulative dosage were associated with chronic renal dysfunction in one study (277). Cyclosporine is associated with a greater risk of CKD than tacrolimus for unclear reasons (8,263).

- Clinical relevance of chronic kidney disease after liver transplantation

Liver transplant recipients with CKD have increased mortality. Stage 4/5 disease has been reported to have a 5 times relative risk of death (8). The association of CKD with cardiovascular mortality is well established (282,283). More recently, a large observational study in the non transplant setting has confirmed increased non cardiovascular death relating to pulmonary disease, cancer and infection (284). However, it is not only mortality that is a relevant measure of worse outcome in CKD. Patients with chronic renal dysfunction also have much greater morbidity, which is evident even in less advanced disease (Table 1.1).

Table 1.1: Morbidity associated with stages of CKD (282,285).

Stage of CKD	Definition	GFR (ml/min/1.73m ²)	Complications
1	Kidney damage with normal eGFR*	≥90	Coronary heart disease
2	Kidney damage with mild ↓ eGFR*	60-89	Hypertension, coronary heart disease
3	Moderate ↓ eGFR	30-59	Hypertension, coronary heart disease, dyslipidaemia, anaemia, impaired nutritional status, bone disease
4	Severe ↓ eGFR	15-29	Hypertension, coronary heart disease, dyslipidaemia, anaemia, impaired nutritional status, bone disease, neuropathy
5	Kidney failure	<15 or on dialysis	Hypertension, coronary heart disease, dyslipidaemia, anaemia, impaired nutritional status, bone disease, neuropathy

* Damage defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.
Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

- Prevention of chronic kidney disease after liver transplantation

At present the management of CKD in the liver transplant population is focussed on CNI minimising strategies. Evidence to support this approach once renal dysfunction is present remains limited and is hampered by heterogeneity between trials with regards type of CNI, trough targets, baseline renal function and time since transplantation, not to mention small patient numbers (Tables 1.2 and 1.3).

Cyclosporine conversion to tacrolimus was associated with no benefit, but no deterioration in renal function in a single uncontrolled study (286). On the other hand, low dose or elimination of CNI with mycophenolate mofetil overall appears to result in some improvement in renal function, and is well tolerated (Table 1.2). The most recent and only negative trial was hampered by significantly different GFRs at baseline between the treatment and control arms (290). An alternative approach has been elimination of CNI with sirolimus (Table 1.3). Yet, Abdelmalek's large study demonstrated no difference in change in renal function 12 months after conversion, and the sirolimus patients had higher rates of biopsy-proven acute rejection and adverse events (294).

Table 1.2: CNi minimisation trials based on addition of mycophenolate mofetil in patients with CKD (287,288,289,290).

Trial	No. patients	Treatment	CNI = Cyclosporine	Years to conversion	Pre conversion GFR	12 month renal function
Beckenbaum, 2009	60 vs 30	MMF + LD CNI vs STD CNI	NA	Range 1-16.6	Mean 39.9	Mean 49.2*
Pageaux, 2006	27 vs 29	MMF + LD CNI vs STD CNI	55.5% vs 58.6%	Median 5.2 vs Median 5.7	Mean 42.6 vs Mean 42.8	Mean 51.7* vs Mean 44.8
Schlitt, 2001	14 vs 14	MMF vs CNI LD	71.4% vs 71.4%	Median 6.3 vs Median 7.5	Mean 49.9 vs Mean 65.3	ΔGFR, +9* vs Δcreat, -6**
Schmeding, 2011	72 vs 70	MMF vs CNI STD	15.7% vs 13.9%	Mean 5.7 vs Mean 4.9	Mean 59.2 vs Mean 70.3****	'No difference' ***

* p<0.05 12 month renal function vs baseline in experimental group but not control

** p<0.05 difference between the 2 groups at 6 months

***No difference in the change in creat or GFR over the followup period. Treatment arm were however more likely to demonstrate improvement in renal function (>20% increase in GFR) and were less likely to demonstrate new impairment. Patients with a baseline creat >106 were 'more likely' to demonstrate an improvement in renal function in the treatment arm.

****Baseline GFR significantly higher in the control arm.

Abbreviations: CKD, chronic kidney disease; CNI, calcineurin inhibitor; GFR, glomerular filtration rate; LD, low dose; MMF, mycophenolate mofetil; STD, standard dose.

Table 1.3: CNI minimisation trials based on addition of sirolimus in patients with CKD (291,292,293,294).

Trial	No. patients	Treatment	Dose sirolimus	Years to conversion	Pre conversion GFR	12 month renal function
Watson, 2007	13	Sirolimus	2mg/day	Median 3.1	Median 49.8	Δ GFR, +6.7*
	vs 14	STD CNI		Median 5.2	Median 47.2	Δ GFR, +0.6
Shenoy, 2007	20	Sirolimus	5mg loading, 3mg/day	Range 0.5-11	Mean 64	Mean 72**
	vs 20	STD CNI		Range 1-12	Mean 60	Mean 58
Eisenberger, 2009	8	Sirolimus	10-15mg loading, 3-5mg/day	Median 4.2	Mean 55.4	Mean 63.0**
	vs 8	STD CNI		Median 2.5	Mean 66.9	Mean 57.9
Abdelmalek, 2012	393	Sirolimus	(10-15mg load)	Mean 4.0	Mean 65.5	Δ GFR, -4.5**
	vs 214	STD CNI	3-5mg/day	Mean 3.8	Mean 65.7	Δ GFR, -3.1

*p<0.05 difference in the change in GFR from baseline to 12-months in treatment arm versus control

** No difference

Abbreviations: CDK, chronic kidney disease; CNI, calcineurin inhibitor; GFR, glomerular filtration rate; STD, standard dose.

In view of the persistence of significant renal dysfunction when CNI minimisation is adopted, interest has shifted towards prevention of renal injury. Four large randomised controlled trials have been published in the last 10 years comparing standard dose with delayed and/or low dose tacrolimus from the immediate peri-operative period (Table 1.4). All of these studies have failed to maintain adequately low CNI trough levels in the treatment arms. Nevertheless, some conclusions can be drawn. Firstly, delayed introduction followed by standard dose tacrolimus with daclizumab cover for the initial post-operative days probably offers no advantage over immediate administration (265,295,296). Although a statistically significant difference was observed in the ReSpECT trial baseline GFR was slightly less in the delayed CNI group (295). Secondly, and encouragingly, long term lower dose tacrolimus from day 1 post transplant does result in less renal function loss (297).

Table 1.4: CNI minimisation trials from day 1 post transplant to prevent CKD (265,295,296,297).

Trial	No. patients	Treatment	Pre transplant GFR	CNI trough levels	12 month renal function
Yoshida, 2005	72 vs 76	Daclizumab, MMF, delayed & LD Tacro	Median 70.6	Not reported	Median 71.7*
		MMF, STD Tacro	Median 73.2	Not reported	Median 73.2
Neuberger, 2009	168 vs 168	Daclizumab, MMF, delayed & LD Tacro	Mean 96.5	Mean 7.4-8.9	ΔGFR, -13.6**
		MMF, LD Tacro	Mean 104.3	Mean 7.8-9.1	ΔGFR, -21.2
		STD Tacro	Mean 101.8	Mean 8.5-11.1	ΔGFR, -23.6
Calmus, 2010	98 vs 101	Daclizumab, MMF, delayed & STD Tacro	Mean SCr 91	Mean 7.5-13	Mean 72.4*
		MMF, STD Tacro	Mean SCr 88	Mean 8.5-14	Mean 71.7
Boudjema, 2011	95 vs 100	MMF, LD Tacro	Median 101	Mean 7.5-12	Mean 90***
		STD Tacro	Median 99	Mean 8-18	Mean 78

* No difference, **p<0.05 Daclizumab, MMF and delayed/LD tacro vs SD tacro, but lower baseline GFR (p=NS), ***p<0.05 treatment vs control
Abbreviations: CNI, calcineurin inhibitor; GFR, glomerular filtration rate; LD, low dose; MMF, mycophenolate mofetil; SCr, serum creatinine; STD, standard dose.

A further 2 trials have examined the benefits of CNI minimisation from 4-12 weeks after transplantation, via the addition of a mammalian target of rapamycin (mTOR) inhibitor (Table 1.5). Tacrolimus elimination with an mTOR inhibitor alone was associated with an increased rate of acute cellular rejection that caused premature termination of the treatment arm (298). However, patients receiving a combination of mTOR inhibitor with reduced dose CNI or mycophenolate mofetil had superior renal function by 12-months post transplant and acceptable rejection rates (298,452). Subsequently published followup data confirms maintained renal benefit to 2-years after transplantation (453). It should be noted that in both the mTOR trials, in contrast to CNI minimisation with mycophenolate mofetil, patients were more likely to discontinue the study drug because of intolerable adverse effects (297,298,452). This is despite a relatively low dose of sirolimus compared to other studies (Table 1.3) (452).

To summarise, CNI minimisation from early post liver transplantation is accompanied by less renal function loss. Nevertheless, 25% of patients still develop CKD (297). Once renal dysfunction occurs it is frequently irreversible. Thus, prevention of post transplant CKD should be a key focus of care, and additional strategies are necessary.

Table 1.5: CNI minimisation trials from 4-12 weeks after transplantation to prevent CKD (298,452).

Trial	No. patients	Treatment	Pre conversion GFR	CNI trough levels	12 month renal function
De Simone, 2012	231 vs 245	Everolimus, Tacro elimination after 4 months	Mean 82.9		ΔGFR, -1.5*
		Everolimus, LD Tacro	Mean 80.8	Mean 6-10.5	ΔGFR, -2.2**
	243	STD Tacro	Mean 78.9	Mean 8-10	ΔGFR, -10.7
Teperman, 2013	149 vs 145	Sirolimus, MMF	Mean 54.3		ΔGFR, +19.7%**
		STD CNI (8% cyclosporine), MMF	Mean 50.6	Mean 7-9	ΔGFR, +1.2%

* Terminated prematurely because of a high incidence of acute cellular rejection, **P<0.05 treatment vs control
Abbreviations: CNI, calcineurin inhibitor; GFR, glomerular filtration rate; LD, low dose; MMF, mycophenolate mofetil; STD, standard dose.

1.5 Conclusion

Renal dysfunction is common and continues to have devastating consequences in patients with all aspects of liver disease. In cirrhosis and ALF, management remains primarily supportive and does not impact on prognosis. Only liver transplantation offers survival benefit, but is a finite resource. Moreover, liver transplantation itself is complicated by both AKI and CKD.

To allow Physicians to help their patients, scientific progress is desperately needed in our understanding of the underlying mechanisms of, and risk factors for, renal dysfunction in liver disease. Only by developing knowledge will preventative strategies and treatment options become available that impact of patient outcome.

1.6 Aims and hypotheses

The principle aim of this thesis is to derive a better understanding of renal dysfunction in liver disease. By doing so, my intention is to move one step closer to improving patient morbidity and mortality in this setting.

The following hypotheses will be addressed:

- In patients with cirrhosis increased activity of endogenous ET-1 is involved in the pathophysiology of renal dysfunction through renal vasoconstriction, and ET-1 antagonism will reverse these effects (Chapter 2).
- In ALF the systemic inflammatory response syndrome is associated with the development of AKI (Chapter 3).
- Estimated GFR is superior to serum creatinine in predicting prognosis in patients on the liver transplant waiting list (Chapter 4).
- Given potentially different pathophysiological mechanisms underlying renal dysfunction, patients transplanted for ALF have comparatively better long term renal outcomes than patients transplanted for CLD (Chapter 5).
- Modifiable patient factors are associated with the long term decline in renal function after liver transplantation (Chapter 6).

CHAPTER 2

Renal dysfunction in cirrhosis: A randomised controlled physiological study of endothelin-1 receptor antagonism in patients with advanced cirrhosis and refractory ascites

2.1 Introduction

Patients with portal hypertension have a spectrum of renal dysfunction that first becomes evident in early compensated cirrhosis, and evolves in parallel with advancing neuro-humoral and circulatory derangement (4). In pre-ascitic cirrhotics there is impaired renal sodium metabolism, with reduced natriuresis in the standing position and following a saline load (61,62). Later, sodium excretion is reduced further, there is a positive sodium balance, and ascites develops (9,64). Finally, a progressive fall in total renal blood flow is eventually accompanied by a fall in GFR when intra-renal compensatory mechanisms fail (88,102). Renal dysfunction is an important prognostic marker in this setting; ascites, low urinary sodium, hyponatraemia and hepatorenal syndrome are consistent predictors of short term mortality in cirrhotic patients (64,299,300).

Endothelin-1 physiology

ET-1 is a potent vasoactive 21 amino acid peptide that has been implicated in the pathophysiology of the renal dysfunction of portal hypertension (301). The endothelin family comprises three isopeptides that are predicted by three separate genes and have distinct tissue distributions, with ET-1 having the principle cardiovascular effects (302,303,304). ET-1 is produced mainly by the endothelium. Other sources of ET-1 include vascular smooth muscle cells, leucocytes, cardiomyocytes, fibroblasts, activated hepatic stellate cells and cholangiocytes, and mesangial cells, podocytes and tubular epithelial cells of the kidney (304,305,306).

The gene product, prepro-ET-1, is cleaved to the precursor molecule, big ET-1, from which ET-1 is generated via the action of endothelin-converting enzyme (ECE) (301,302). Gene transcription of ET-1 is increased by several factors relevant to CLD, including low sheer stress, angiotensin II, adrenaline and inflammatory cytokines, and reduced by nitric oxide, prostaglandins and natriuretic peptides (304).

ET-1 acts in an autocrine and paracrine manner on 2 distinct G-protein-coupled-receptor subtypes, Endothelin-A (ET-A) and Endothelin-B (ET-B) (307,308,309). The binding of ET-1 to ET-A, and to a lesser extent ET-B, receptors of the vascular smooth muscle mediates vasoconstriction, whilst in the endothelium ET-1 via ET-B receptors mediates vasodilatation through the release of nitric oxide and prostacyclin (304). Vascular ET-B receptors also act as an important route of clearance of ET-1 from the circulation (310,311,312). Hence, ET-B receptor antagonism is associated with an elevated plasma concentration of ET-1, which may in turn increase ET-A receptor activation (311,312).

Systemic haemodynamic effects of endothelin-1

In healthy humans, brachial artery infusion of phosphoramidon, an inhibitor of ECE, and BQ-123, a selective ET-A receptor antagonist, causes local vasodilatation supporting a role for ET-1 in the maintenance of basal vascular tone (313). Systemic ET-1 blockade is associated a reduction in peripheral vascular resistance and blood pressure (314). Such effects are similar following BQ-123 infusion and combined ET-A and B receptor antagonism confirming that the physiological consequence of

ET-1 is predominantly mediated via the ET-A receptor (315). ET-B receptor antagonism with BQ-788 causes an increase in systemic vascular resistance (315). Therefore, the overall effect of ET-B receptor activation on the circulation in health appears to be that of vasodilatation (315).

Renal haemodynamic effects of endothelin-1

The kidney has a high concentration of ET-1 and contains abundant ET-1 receptors (316,317,318). In humans, the ET-B receptor subtype predominates with an ET-B to ET-A ratio of 2:1 (319). ET-A receptors are localised to vascular smooth muscle of the arcuate arteries, arterioles, glomeruli and vasa recta (319). ET-B receptors are found on the endothelium of large arteries at the cortico-medullary junction and microvasculature, plus the epithelial cells lining the renal tubule especially the collecting duct (319). Via these receptors ET-1 modulates renal blood flow and intra-renal haemodynamics, and tubular function, and has pro-inflammatory and pro-fibrotic effects.

Exogenous ET-1 results in an increase in renal vascular resistance, fall in total renal blood flow and reduction in GFR (312,320,321). These changes are abolished by co-infusion of BQ-123, thereby implying that renal vasoconstriction is largely mediated by the ET-A receptor subtype (321). The renal microcirculatory effects in humans are not currently known. Animal models suggest that ET-1 administration is associated with arteriole vasoconstriction, which is more pronounced in the afferent than efferent arterioles. Afferent vasoconstriction is mediated by both ET-A and ET-

B receptor activation. In the efferent arteriole ET-B receptors are more prominent, but their overall contribution to resting tone is less clear and may be model specific. Within the glomerulus itself, ET-1 may influence the GFR through mesangial cell contraction via ET-A, and potentially by cytoskeletal remodelling in podocytes (322).

Human antagonist studies do not support a role for ET-A receptors in the maintenance of renal vascular tone in health. B-123 infusion has no effect on renal haemodynamics or GFR (312,315,321,323,324,325,326). Combined ET-A and ET-B receptor antagonism with BQ-123 and BQ-788 similarly did not alter total renal blood flow or renal vascular resistance, although the administration of the mixed ET-A/B receptor antagonist SB 209670 precipitated an increase in total renal plasma flow (315,327). BQ-788 infusion alone is associated with renal vasoconstriction and a fall in total renal blood flow suggesting that ET-1 via ET-B receptor mediated vasodilatation is relevant to the maintenance of renal vascular tone (315).

Renal tubular effects of endothelin-1

ET-1 is also important in modulating sodium and water reabsorption within the renal tubule. Although the predominant effects are in the collecting duct, ET-1 is expressed and has actions in other regions. In the proximal tubule the effects appear to be mainly via the ET-B receptor, which both stimulates and inhibits sodium transport processes depending on ET-1 concentrations and degree of acidosis. In the

thick ascending limb only ET-B receptors have been detected and their activation results in reduced sodium and chloride reuptake (322).

The collecting duct, and in particular the inner medullary collecting duct, is the main site of ET-1 synthesis in the kidney (328,329). Here, production is increased in response to extra-cellular fluid volume expansion, as well as other factors such as local hypoxia, IL-1 β and transforming growth factor- β , and reduced by lower pH and interferon- γ (322). Moreover, the collecting duct is the major site of ET-1 receptor expression with the ET-B receptor being the principal isoform (318,330,331,332). In vitro and knock out murine models provide strong evidence that ET-1 is an important inhibitor of collecting duct sodium reabsorption (333,334,335,336). ET-1 inhibits sodium uptake via the epithelial sodium channel, involving both ET-B receptor mediated mitogen activated-protein kinase (MAPK) activation and nitric oxide (322). In addition, ET-B receptor activation inhibits collecting duct water reabsorption through inhibition of arginine vasopressin-stimulated osmotic water permeability (322). The role of the ET-A receptor in sodium and water transport in the collecting duct remains unclear.

Despite well documented ET-1 induced natriuresis in experimental models, selective ET-A, selective ET-B and combined ET-A/B receptor antagonism in healthy volunteers has not been associated with any change in urinary sodium excretion or fractional excretion (315,337). However, in the setting of angiotensin-converting enzyme inhibition, BQ-123 infusion resulted in an ET-B receptor dependent

natriuresis that was mediated by nitric oxide and, to a lesser degree, prostanoids (325).

The role of endothelin-1 in the pathophysiology of renal disease

There is compelling animal data supporting a role for ET-1 in the pathophysiology of acute and chronic renal disease (338,339). In humans with CKD, acute selective ET-A receptor antagonism was associated with renal vasodilatation with an increase in total renal blood flow, although no change in GFR. The fall in effective filtration fraction (EFF) and proteinuria suggested a greater effect on efferent arteriole tone and reduced glomerular pressure. Selective ET-B receptor antagonism resulted in renovasoconstriction and decreased GFR, but combined ET-1 receptor blockade had no renal haemodynamic effects. No changes in urinary sodium excretion or fractional excretion were observed (315). In another study of patients with non diabetic proteinuric CKD, ET-A receptor inhibition produced a marked natriuresis (340). Longer term, selective ET-A receptor antagonism has been found to reduce the EFF, GFR and proteinuria in non diabetic CKD, and to reduce proteinuria in diabetics (340,341,342). Finally, in patients with chronic renal insufficiency undergoing cardiac angiography combined ET-1 blockade resulted in an increased incidence of radiocontrast nephrotoxicity supporting a protective effect (343).

Evidence supporting a role for endothelin-1 in the pathophysiology of portal hypertension

Patients with cirrhosis demonstrate elevated plasma ET-1 levels, and concentrations increase with rising Child Pugh score, and are higher in patients with ascites compared with compensated disease, and in patients with hepatorenal syndrome compared with those with maintained renal function (344,345). The parallel increase in plasma big ET-1 concentration is consistent with an increase in ET-1 synthesis (346). Greater hepatosplanchnic release has been observed suggesting that the liver is a primary source (347). Moreover, ET-1 expression is markedly enhanced in human cirrhotic liver tissue with activated hepatic stellate cells being identified as the major site of synthesis (348). Other apparent important sources of increased ET-1 production in these patients are the spleen and kidney (346,349). Reduced hepatic clearance of ET-1 also contributes (350). Splanchnic vasodilatation, regional hypoxia, increased circulating vasoactive mediators and endotoxin are likely mechanisms underlying the increased ET-1 release (304,351).

ET-1 has been implicated in the pathophysiology of portal hypertension. In cirrhosis, activated hepatic stellate cells, which are known to regulate sinusoidal resistance to blood flow, demonstrate increased expression of ET-A and ET-B receptors (352,353). ET-1 stimulates contraction of stellate cells and the hepatic sinusoid, and has been shown to increase intrahepatic portal vascular resistance (354,355,356,357). ET-A receptor antagonism and ET-B receptor antagonism in cirrhotic rats resulted in sinusoidal dilatation and reduced portal pressure, and sinusoidal constriction and

increased portal pressure, respectively (358). Combined antagonism reduced portal pressure (358). Therefore, it is postulated that the hepato-splanchnic production of ET-1 contributes to portal hypertension by mediating intrahepatic stellate cell contraction and an increase in hepatic sinusoidal tone (358).

The systemic infusion of ET-A, ET-B and non selective ET-1 receptor antagonists in humans has not been demonstrated to alter the hepatic venous pressure gradient, perhaps as a consequence of competing effects on intrahepatic, collateral and splanchnic circulations (359,360). Nevertheless, forearm haemodynamic studies do support the presence of an activated ET-1 system with a greater contribution to the maintenance of peripheral basal vascular tone via the ET-A receptor in preascitic cirrhosis than healthy controls (361,362). Interestingly, Vaughan et al found that in patients with ascites local ET-1 infusion was associated with forearm vasodilatation rather than constriction, raising the possibility that the ET-B receptor plays a more prominent role in these patients (363). Yet, there were significant methodological problems with the study that limit its interpretation (364).

Evidence supporting a role for endothelin-1 in the pathophysiology of portal hypertension-related renal dysfunction

Given the described observations in non cirrhotic experimental and clinical studies, and the evidence for increased activity in portal hypertension, ET-1 is an attractive candidate to explain the altered renal haemodynamic and tubular function of portal hypertensive-related renal dysfunction.

In support of this hypothesis, plasma ET-1 levels correlate negatively with creatinine clearance and effective renal plasma flow (ERPF) (345,365). Furthermore, an acute rise in portal pressure by TIPSS occlusion is accompanied by an acute increase in arterial ET-1 concentration and renal ET-1 production, and a dramatic reduction in renal plasma flow (366). Therefore, ET-1 may be a mediator of the hepato-renal reflex. In patients with hepatorenal syndrome, liver transplantation is associated with a rapid decrease in ET-1 concentrations and subsequent improvement in renal function, suggesting a causal role (367).

There are currently no ideal animal models of portal hypertensive renal dysfunction. Galactosamine-induced hepatotoxicity resembles acute hepatic failure and is associated with functional renal impairment. In this model, ET-A receptor expression was increased in the renal cortex, and a non selective ET-1 receptor antagonist prevented the development of renal failure in the absence of any effect on total renal blood flow or renal vascular resistance (201). Nevertheless, the results may not be applicable to hepatorenal syndrome because the renal dysfunction of acute liver failure and portal-hypertensive renal dysfunction may not be comparable. Rats with carbon-tetrachloride-induced cirrhosis have upregulation of ET-B receptors in the inner-medullary collecting duct, and combined ET-1 receptor blockade resulted in decreased water excretion that was not observed in controls (368). However, the renal dysfunction in this setting likely represents direct drug-induced nephrotoxicity (369). Most recently, in a non cirrhotic model of portal hypertension that was associated with a reduction in renal perfusion pressure, no change in ET-1 receptor expression was noted (370).

In patients, there are no placebo controlled studies examining the role of ET-1 in the pathophysiology of portal hypertensive renal dysfunction. Soper observed in 3 patients with alcoholic cirrhosis, and reduced renal plasma flow and GFR, an increase in both parameters following BQ-123 infusion (371). On the other hand, combined ET-A and ET-B receptor antagonism in 5 patients with cirrhosis and type 2 hepatorenal syndrome was not accompanied by any improvement in renal function. Instead, 4 patients demonstrated a rise in serum creatinine and there was a statistically significant fall in 24-hour urinary volume (372). No conclusions can be drawn from these observational studies and the question remains whether ET-1 receptor antagonism has a potential therapeutic role in portal hypertensive related renal dysfunction.

2.2 Aims

We hypothesised that the increased activity of endogenous ET-1 is involved in the pathophysiology of renal dysfunction in patients with advanced liver disease through renal vasoconstriction, and that ET-1 antagonism would reverse these effects, increase renal blood flow and improve renal function. The aim of this study was to examine the systemic haemodynamic and renal effects of ET-1 antagonism in patients with advanced cirrhosis and refractory ascites and/or hepatorenal syndrome.

2.3 Methods

This was a randomised, double-blind, placebo-controlled crossover study. Five patients with advanced cirrhosis and refractory ascites were recruited. Four patients attended for 3 separate study periods: (A) selective ETA receptor antagonism; (B) combined ETA/B receptor antagonism; (C) saline placebo. The fifth patient withdrew after participating in 1 study period only.

The studies were performed in a quiet temperature-controlled room with the subject recumbent throughout in the Royal Infirmary of Edinburgh Clinical Research Facility between April 2007 and October 2008. The study protocol was approved by the Local Research Ethics Committee and all subjects provided written informed consent. The investigations conformed to the principles outlined in the Declaration of Helsinki.

Subjects

Patients had advanced cirrhosis and refractory ascites as defined by the International Ascites Club (4). Suitable inpatients and outpatients were recruited from the Centre for Liver and Digestive Disorders at The Royal Infirmary of Edinburgh. The exclusion criteria were as follows: below the age of consent or mentally or legally incapacitated; enrolled in another trial; malignancy; TIPSS in situ; pre-existing renal disease other than hepatorenal syndrome; significant comorbidity (diabetes mellitus, heart or lung disease, peripheral vascular disease, musculoskeletal or other condition

that prevents the subject from lying supine for prolonged time periods); taking vasoactive medications; on anti-coagulant medications; prothrombin ≥ 17 seconds and/or platelets $< 50 \times 10^9/l$; active sepsis or other SIRS such as acute alcoholic hepatitis. Prior to the study patients had underwent full clinical assessment including blood and urine chemistry and renal ultrasonography to exclude intrinsic kidney disease.

Healthy control data was provided by Goddard et al from a study of ET receptor antagonism in health and CKD (315). In this study, with an identical study protocol performed by grant co-applicants and members of the present study group, 8 healthy volunteers were recruited. The exclusion criteria included the use of any medications within the preceding 2 weeks.

Primary outcome measures

The primary outcome measures were effective renal blood flow (ERBF) and GFR measured by standard para-aminohippurate sodium (PAH) and inulin clearance techniques, respectively.

Drugs

BQ-123 (Clinalfa AG and American Peptide Company, see Limitations Chapter), a selective ETA receptor antagonist (373), was infused at 100 nmol/l for 15 minutes and at 1000 nmol/l for 15 minutes 90 minutes later. These doses were selected from a

previous study as having a threshold and maximum haemodynamic effect in healthy controls (374). BQ-788 (Clinalfa AG and American Peptide Company, see Limitations Chapter), a selective ETB receptor antagonist (375), was infused at 30 and 300 nmol/l for 15 minutes, doses shown to be haemodynamically active in a previous systemic dose ranging study (376). Drugs were dissolved in physiological saline (0.9%; Baxter Healthcare Ltd) and infused intravenously at a constant rate of 1 ml/min. Saline was administered as placebo.

PAH (Clinalfa AG and Merck, see Limitations Chapter) and inutest (Serb) were dissolved in dextrose 5% (Baxter) and administered as a bolus loading dose of 0.4 g PAH and 3.5 g inutest in 100 ml dextrose over 15 minutes followed by a maintenance infusion of 792 mg PAH (13.2 mg/min) and 1200 mg inulin (20 mg/min) administered in 120 ml dextrose/hr.

All drugs were prepared by a research nurse unconnected with the study.

Assays

At prespecified time points, venous blood was collected into Lithium heparin tubes for measurement of PAH and inulin, and EDTA tubes for haematocrit (Hct). Similarly, urine was collected in universal containers for the measurement of PAH and inulin.

Hct was measured on whole blood with a Coulter counter. All other blood samples were centrifuged immediately at 1000 xg (2500-3000 rpm) at 4 °C for 20 minutes and subsequently stored along with the urine in a -80 °C freezer to allow batch processing at a later stage.

Inulin was determined by spectrophotometry after hydrolysis to fructose, and PAH was determined by high-performance liquid chromatography.

Pre study conditions

Subjects were requested to adhere to a low sodium diet (100 ml/day) to standardise salt intake and to avoid caffeine, nicotine and alcohol for 72 hours prior to each study. They fasted from midnight the night before and during the study, other than a light breakfast that was provided on arrival in the Clinical Research Facility. Diuretic medications were omitted on the days of participation.

Study protocol

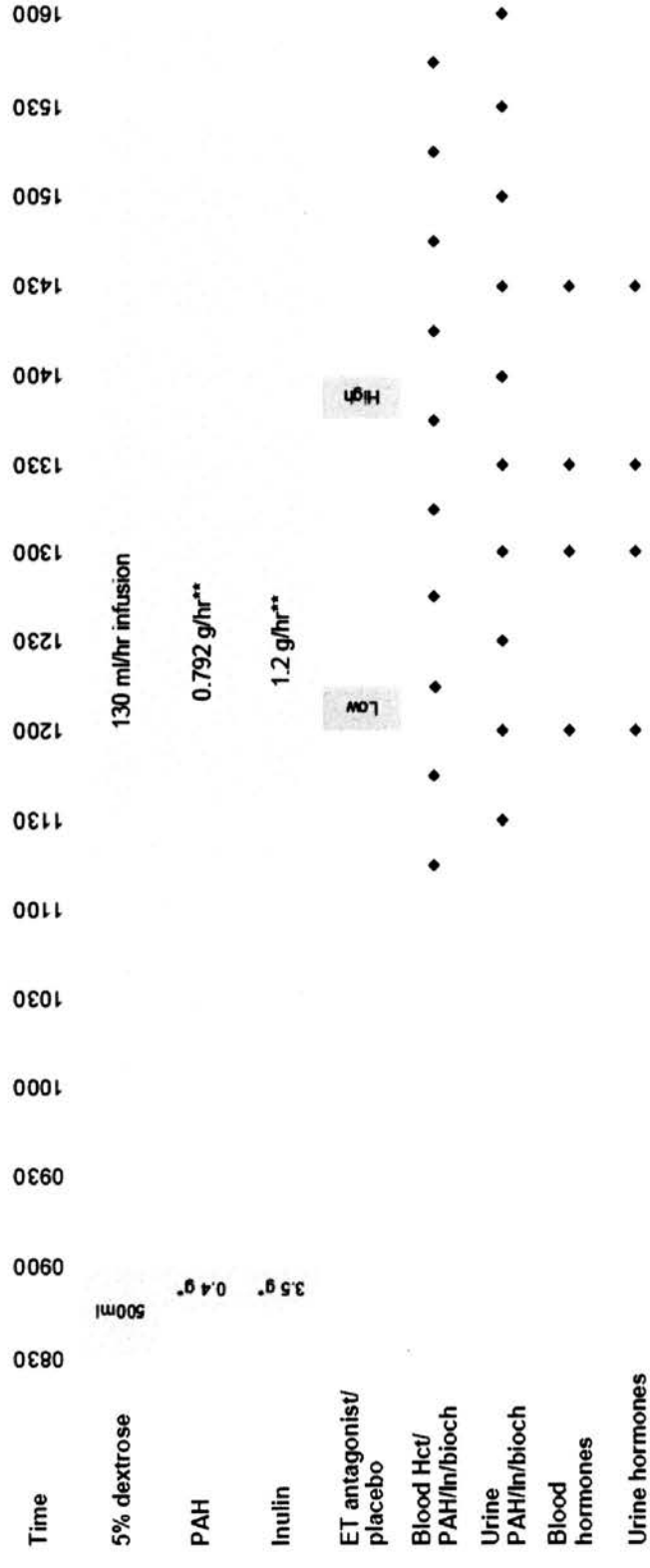
Subjects each attended for 3 study periods in a randomised manner: (A) selective ETA receptor antagonism with BQ-123 100 nmol/min and 1000 nmol/min; (B) combined ETA/B receptor antagonism with BQ-123/BQ-788 100/30 nmol/min and 1000/300 nmol/min; (C) saline placebo. The study periods were separated by at least 7 days because previous studies with the same doses of BQ-123 and BQ-788 have

demonstrated that haemodynamic changes return to baseline after 4 hours, to ensure complete washout of the study drugs (374,376).

The study protocol is outlined in Figure 2.1. On each study day, an 18 gauge cannula was sited into an antecubital vein in each arm and a urinary catheter was inserted. Diuresis was induced by 500 ml of 5% dextrose over 30 minutes through the left arm cannula followed by 130 ml/hr maintenance infusion. Fifteen minutes later, through the same cannula, the bolus loading dose of PAH and inulin was co-administered in 100 ml 5% dextrose over 15 minutes followed by the maintenance dose co-administered in 120 ml/hr 5% dextrose. The maintenance infusions were continued to study end. After a minimum 2-hour equilibration period (the equilibration period was extended if the last 2 urinary flow rates were not within 25% of each other), 2 sets of baseline measurements were performed. The low dose ET-1 antagonist/placebo was then administered through the right antecubital cannula, and the high dose ET-1 antagonist/placebo administered 90 minutes later.

Systemic haemodynamic measurements (heart rate; blood pressure; cardiac index) were performed at 15 minute intervals from one hour prior to administration of the low dose study drug until study end using validated non-invasive automated techniques (377,378). Urinary flow rate (UFR)s were determined from 30 minute urine collections following catheter insertion. Urine was collected for sample analysis from -30 minutes (PAH, inulin). Blood was sampled at the mid point of each collection (PAH, inulin, Hct).

Figure 2.1: Study protocol. UFR performed every 30 minutes from 0900. Systemic haemodynamic measurements performed at 0830 and every 15 minutes from 1100. * bolus loading dose of 0.4 g PAH and 3.5 g Inulin co-administered in 100 ml 5% dextrose over 15 minutes. ** maintenance infusion of 0.792 g PAH (13.2 mg/min) and 1.2 g inulin (20mg/min) co-administered in 120 ml 5% dextrose per hour. In total during maintenance patients received 250 ml 5% dextrose per hour.



Abbreviations: Hct, haematocrit; In, Inulin; PAH, para-aminohippurate sodium; UFR, urinary flow rate.

Statistical analyses

Blood pressure at each time point was calculated as the mean of 2 recordings and represented as mean arterial pressure ($\text{MAP} = \text{diastolic blood pressure} + 1/3 \text{ pulse pressure}$). Bioimpedance data at each time point was calculated as the mean of 4 recordings, each the average of 15 consecutive heart beats. Data was corrected for body surface area to give cardiac index, for direct comparison between subjects ($\text{cardiac index} = \text{cardiac output} / \text{body surface area}$). Systemic vascular resistance index (SVRI) was calculated by dividing MAP by cardiac index and expressed in $\text{dyne.s m}^2 / \text{cm}^5$. ERPF and GFR were calculated from PAH and inulin clearances, respectively (379). GFR was adjusted for body surface area. ERBF was calculated by dividing ERPF by (1-Hct), effective renal vascular resistance (ERVR) by dividing MAP by ERBF, and EFF by dividing GFR by ERPF $\times 100\%$.

Baseline data were calculated as the mean of the 2 time points that immediately preceded administration of the first study drug. Statistical analysis was performed on untransformed data. Two comparisons of interest were preidentified as placebo versus BQ-123 and placebo versus BQ-123/788. Responses were examined by repeated-measures ANOVA with Bonferroni correction was used to assess significance at specific time points. $P < 0.05$ was considered statistically significant.

Data was analysed using the SPSS 18 package. All values are expressed as mean \pm standard error of the mean (SEM).

2.4 Results

Baseline demographics of the patients are outlined in table 2.1. All four patients had advanced liver cirrhosis with a mean Child Pugh score of 11 and Model for End-stage Liver Disease (MELD) score of 15. All had refractory ascites and had undergone large volume paracentesis within the preceding 4 weeks. The mean serum sodium was 134 mmol/l and the mean serum creatinine was 90 μ mol/l.

No patient was receiving vasoactive medications at the time of participation in the study. The mean spironolactone dose and frusemide dose was 200 (range 100-300) mg and 20 (range 0-40) mg, respectively.

Baseline systemic haemodynamics and renal function

Baseline systemic haemodynamics and renal function are outlined in table 2.2. Patients demonstrated the typical circulatory changes of portal hypertension. When compared to healthy controls, patients had a lower mean MAP ($p=0.003$) and SVRI ($p<0.001$), and higher mean cardiac index ($p<0.001$) and heart rate ($p<0.001$). Patients had a similar mean ERBF ($p=0.621$) and ERVR ($p=0.400$) to controls (315). Mean GFR ($p=0.003$) and EFF ($p<0.001$) were lower in the patient group.

Table 2.1: Patient demographics.

Clinical characteristics	Patient N=4	Control N=8	P value
Age (years)	55+/-4 (49-65)	47+/-5 (23-64)	0.325
Gender			
Male:Female	2:2		
Aetiology of cirrhosis			
Alcohol:Hepatitis C	2:2		
Severity of liver disease:			
Bilirubin ($\mu\text{mol/l}$)	43+/-9 (25-64)		
International normalised ratio	1.4+/-0.1 (1.1-1.5)		
Albumin (g/L)	28+/-2 (23-33)		
MELD score	15+/-2 (10-17)		
UKELD score	55+/-2 (51-58)		
Child Pugh score	11+/-1 (9-12)		
Measures of renal function:			
Refractory ascites	4		
Serum sodium (mmol/l)	134+/-2 (131-139)		
Urea (mmol/l)	4.4+/-1.6 (1.0-8.3)		
Creatinine ($\mu\text{mol/l}$)	90+/-17 (62-137)	85+/-5 (62-111)	0.719
MDRD 6 eGFR (ml/min/1.73m^2)	75+/-12 (45-97)		
Body mass index (kg/m^2)	26+/-2 (22-31)	26+/-2 (18-31)	1.000
Active alcohol excess	0		
Smoker	2		

Values expressed as mean +/- standard error of the mean (range).

Abbreviations: MDRD 6 eGFR, Modification of Diet in Renal Disease 6-variable estimated glomerular filtration rate; MELD, Model for End Stage Liver Disease; UKELD, UK score for Patients with End-Stage Liver Disease

Table 2.2: Baseline haemodynamics and renal function.

	MAP (mmHg)	SVRI (dyne.s m ² /cm ⁵)	Cardiac index (l/min/m ²)	Heart rate (bpm)	GFR (ml/min/1.7 3m ²)	ERBF (ml/min)	ERVR (mmHg.mi n/l)	EFF (%)	UFR (ml/min)
Patient 1	85	1428	4.8	65	61	825	104	11.1	3.3
Patient 2	85	1027	6.7	91	75	722	118	14.1	1.3
Patient 3	77	1308	4.7	90	43	590	136	10.4	3.7
Patient 4	77	1304	4.9	73	31	459	168	8.7	5.2
Patient Mean (SEM) n=4	81 (2)	1267 (58)	5.3 (0.3)	80 (4)	53 (5)	649 (45)	132 (8)	11.1 (0.7)	3.4
Control Mean (SEM) n=8	94 (2)	3089 (269)	2.6 (0.3)	58 (2)	116 (11)	683 (41)	148 (12)	25.4 (2.0)	-
P value	0.003	<0.001	<0.001	<0.001	0.003	0.621	0.400	<0.001	-

Abbreviations: EFF, effective filtration fraction; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; GFR, glomerular filtration rate; MAP, mean arterial pressure; SEM, standard error of the mean; SVRI, systemic vascular resistance index; UFR, urinary flow rate.

Systemic haemodynamic and renal effects of placebo

In patients, placebo was not associated with any change in MAP (83 (4) vs 85 (5) mmHg; mean (SEM); $p=0.284$), SVRI (1318 (82) vs 1391 (150) $\text{dyne.s m}^2/\text{cm}^5$; mean (SEM); $p=0.576$), cardiac index (5.1 (0.4) vs 5.0 (0.4) l/min/m^2 ; mean (SEM); $p=0.816$) or heart rate (80 (7) vs 75 (5) bpm; mean (SEM); $p=0.284$) from baseline to study end.

Placebo was associated with an increase in ERVR (132 (16) vs 180 (26) mmHg.min/l; mean (SEM); $p=0.024$) and reduction in ERBF (659 (102) vs 500 (81) ml/min; mean (SEM); $p=0.016$), although no change in GFR (50 (9) vs 47 (8) ml/min/ 1.73m^2 ; mean (SEM); $p=0.668$). There was a trend towards an increase in EFF (10.3 (0.9) vs 12.7 (0.8) %; mean (SEM); $p=0.050$). There was no effect on UFR (3.4 (0.7) vs 3.6 (0.6) ml/min; mean (SEM); $p=0.712$), therefore negating any waning effect of diuretic therapy.

Placebo had similar systemic haemodynamic effects in healthy controls (315).

Systemic haemodynamic effects of endothelin-1 receptor antagonism

In patients, the administration of BQ-123 and BQ-123/788 had no statistically significant effect on MAP, SVRI, cardiac index or heart rate (Table 2.3, Figure 2.2).

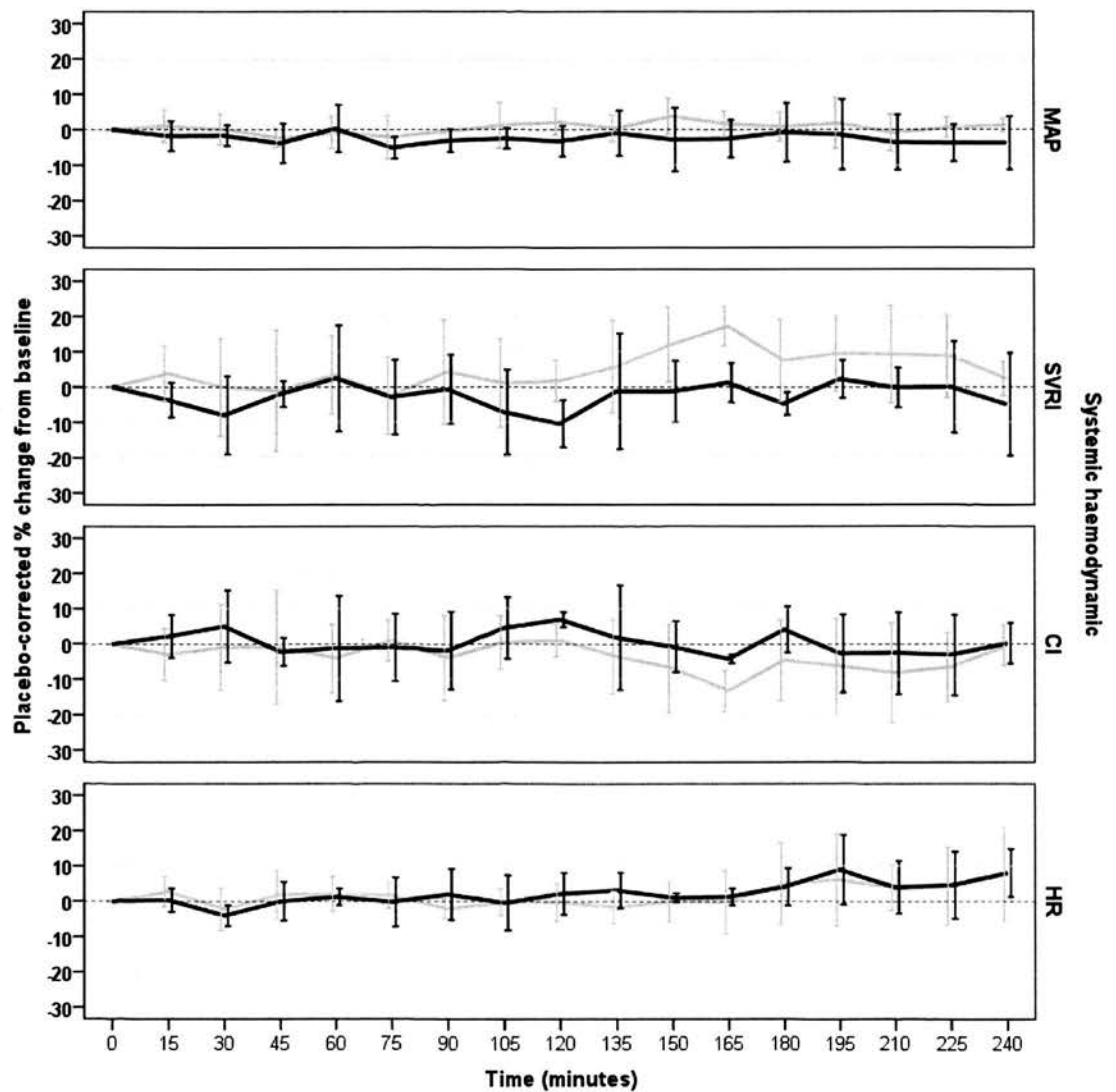
In healthy controls, BQ-123 and BQ-123/788 reduced MAP (BQ-123 -4 ± 1 mmHg, $p<0.01$; BQ-123/788 -4 ± 2 mmHg, $p<0.01$; peak mean placebo-corrected change from baseline) and SVRI (BQ-123 -591 ± 104 dyne.s m^2/cm^5 , $p<0.01$; BQ-123/788 -498 ± 159 dyne.s m^2/cm^5 , $p<0.01$), and was associated with an increase in cardiac index (315).

Table 2.3: Peak placebo-corrected change from baseline following ET-1 receptor antagonism.

	MAP (mmHg)	SVRI (dyne.s m ² /cm ⁵)	Cardiac index (l/min/m ²)	Heart rate (bpm)	GFR (ml/min/1.7 3m ²)	ERBF (ml/min)	ERVR (mmHg.min /l)	EFF (%)	UFR (ml/min)
BQ-123	+3	+185	-0.7	+7	-11	-143	+46	+1.0	-3.5
%	+3.8	+17.2	-13.2	+7.7	-17.4	-21.8	+34.0	+15.3	-156.1
P value	0.184	0.225	0.194	0.205	0.106	0.061	0.186	0.571	0.190
BQ-123/788	-4	-140	+0.4	+8	-11	+119	-39	-1.9	-3.0
%	-5.1	-10.4	+7.0	+9.0	-18.5	+20.9	-25.7	-20.0	-138.0
P value	0.218	0.182	0.280	0.129	0.012	0.145	0.066	0.101	0.240

Abbreviations: EFF, effective filtration fraction; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; ET-1, endothelin-1; GFR, glomerular filtration rate; MAP, mean arterial pressure; SVRI, systemic vascular resistance index; UFR, urinary flow rate.

Figure 2.2: Systemic haemodynamics after ET-1 receptor antagonism. Values expressed as mean placebo-corrected % change from baseline \pm SEM. Grey line, BQ-123; black line, BQ-123/788. No statistically significant differences observed.



Abbreviations: CI, cardiac index; ET-1, endothelin-1; HR, heart rate; MAP, mean arterial pressure; SEM, standard error of the mean; SVRI, systemic vascular resistance index.

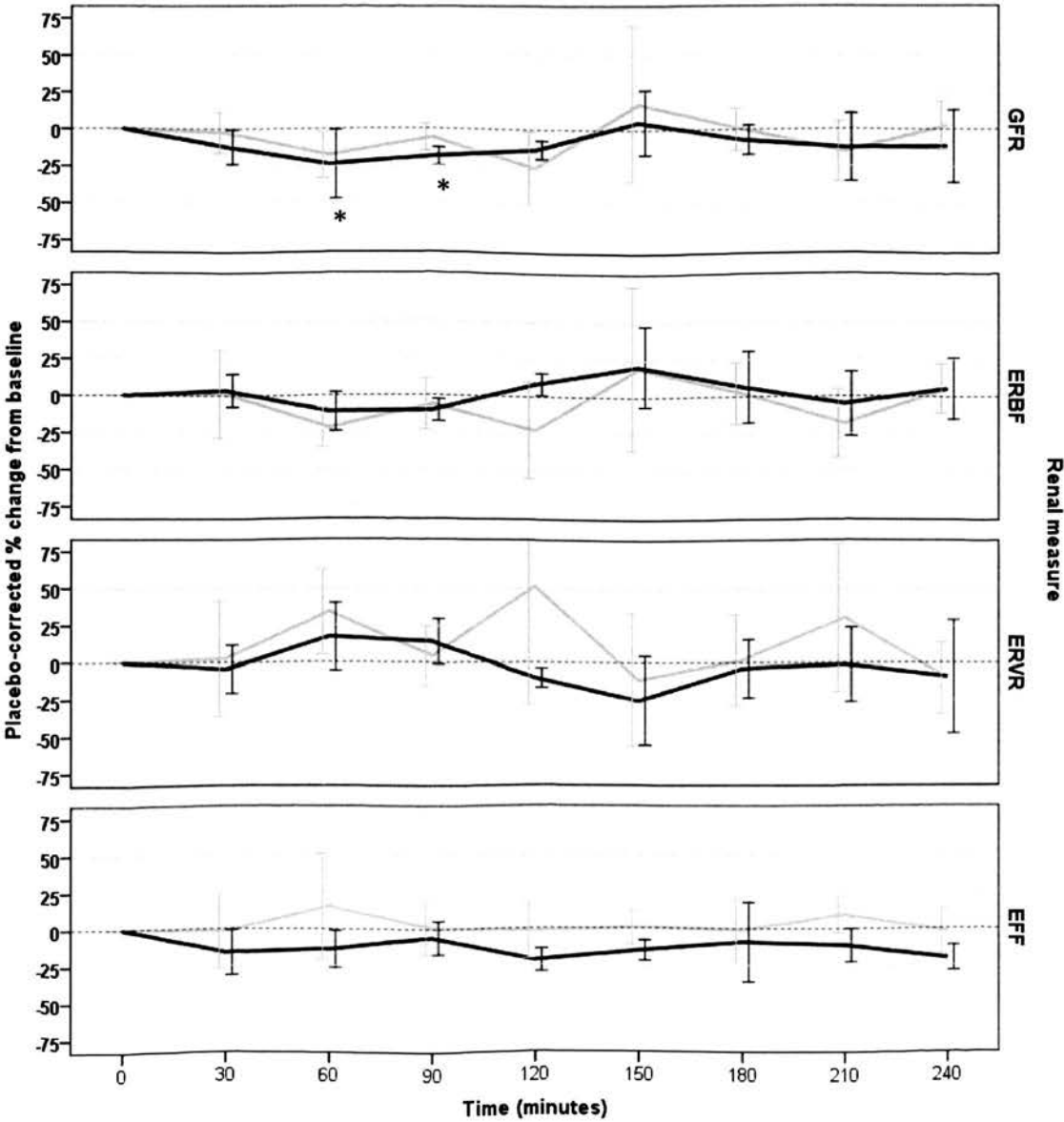
Renal effects of endothelin-1 receptor antagonism

BQ-123 alone did not result in any statistically significant change in patient renal function (Table 2.3, Figure 2.3). BQ-123/788 reduced GFR in a non-dose dependent manner ($-11 \text{ ml/min/1.73m}^2$, $p=0.012$; peak mean placebo-corrected change from baseline), but had no effect on ERBF or ERVR.

When individual patient data was considered, the UFR of Patient 2 was observed to swing dramatically and could not be explained physiologically (placebo $+470 \%$; BQ-123 $+270 \%$; BQ-123/788 $+716 \%$; peak $\%$ change from baseline) (Figure 2.4). The statistical analysis of renal function following ET-1 receptor antagonism was therefore repeated excluding Patient 2 (Table 2.4, Figure 2.5). In this reanalysis, BQ-123 did not result in any statistically significant change in renal function. BQ-123/788 did not alter ERBF or ERVR, but reduced GFR ($-8 \text{ ml/min/1.73m}^2$, $p=0.016$; peak mean placebo-corrected change from baseline), EFF (-2.1% , $p=0.029$) and UFR (-1.3 ml/min , $p=0.009$).

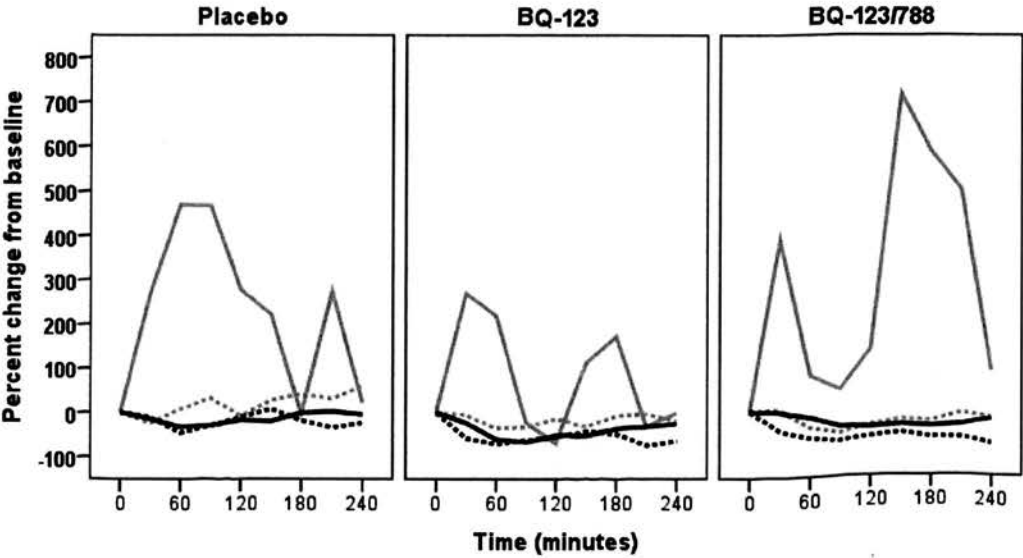
Neither BQ-123 nor BQ-123/788 altered renal function of healthy controls (315).

Figure 2.3: Renal function after ET-1 receptor antagonism. Values expressed as mean placebo-corrected % change from baseline \pm SEM. Grey line, BQ-123; black line, BQ-123/788. * $p < 0.05$.



Abbreviations: EFF, effective filtration fraction; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; ET-1, endothelin-1; GFR, glomerular filtration rate; SEM, standard error of the mean.

Figure 2.4: Individual patient UFR following placebo and ET-1 receptor antagonism. Grey broken line, Patient 1; grey solid line, Patient 2; black broken line, Patient 3; black solid line, Patient 4.



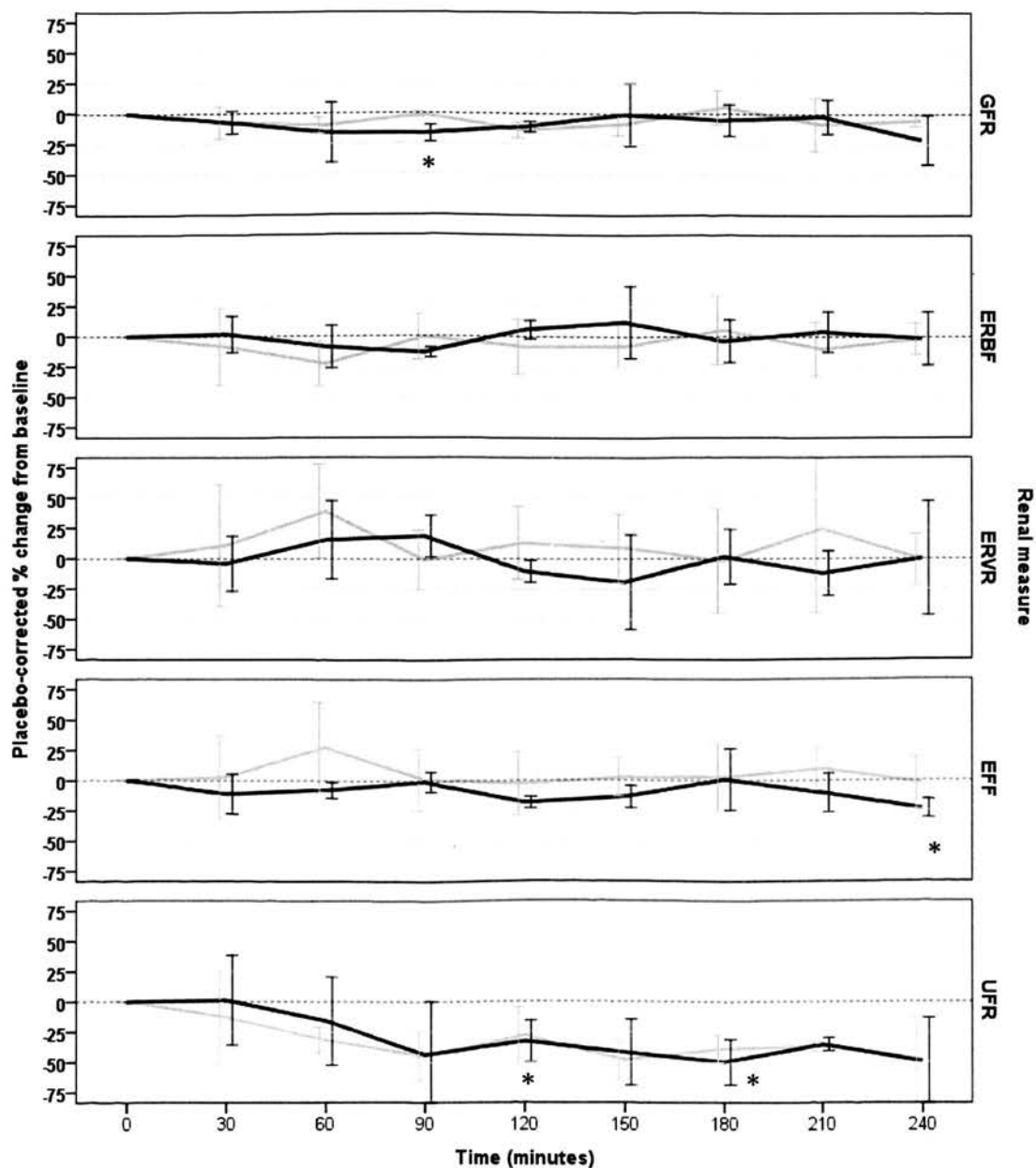
Abbreviations: ET-1, endothelin-1; UFR, urinary flow rate.

Table 2.4: Peak placebo-corrected change from baseline following ET-1 receptor antagonism (patient 2 excluded from analysis).

	GFR (ml/min/1.73 m ²)	ERBF (ml/min)	ERVR (mmHg.min/l)	EFF (%)	UFR (ml/min)
BQ-123	-6	-143	+54	+2.4	-1.9
%	-13.6	-22.5	+39.1	+27.6	-48.3
P value	0.149	0.095	0.238	0.107	0.060
BQ-123/788	-8	-76	+25	-2.1	-1.3
%	-16.7	-13.7	+18.7	-23.4	-33.0
P value	0.016	0.415	0.196	0.029	0.009

Abbreviations: EFF, effective filtration fraction; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; ET-1, endothelin-1; GFR, glomerular filtration rate; UFR, urinary flow rate.

Figure 2.5: Renal function after ET-1 receptor antagonism (excluding Patient 2).
 Values expressed as mean placebo-corrected % change from baseline \pm SEM.
 Grey line, BQ-123; black line, BQ-123/788. * $p < 0.05$.



Abbreviations: EFF, effective filtration fraction; ERBF, effective renal blood flow; ERVR, effective renal vascular resistance; ET-1, endothelin-1; GFR, glomerular filtration rate; UFR, urinary flow rate.

2.5 Discussion

This is the first placebo controlled study of the renal effects of ET-1 antagonism in patients with advanced cirrhosis and manifestations of portal hypertension-related renal dysfunction. We have shown that in patients with refractory ascites, acute combined ET-A and ET-B receptor blockade resulted in a fall in GFR despite no change in systemic haemodynamics or total renal blood flow, and a marked reduction in UFR. These findings negate our hypothesis and are consistent with a reno-protective role for ET-1 in portal hypertension-related renal dysfunction.

The fall in GFR was in the setting of a reduction in EFF, thus suggesting that non selective ET-1 receptor antagonism had a greater effect on efferent arteriole tone with a fall in glomerular pressure. Given that these observations were only made in the presence of ET-B receptor antagonism, we postulate that ET-B mediated efferent arteriole vasoconstriction is important in maintaining glomerular perfusion pressure in these patients. In agreement, several in vitro animal models have demonstrated a significant vasoconstrictor function of ET-B receptors in the post glomerular arteriole (380,381,382). During ET-A receptor blockade a progressive increase in ET-1 dose precipitated initial vasodilatation, followed by vasoconstriction (96). Therefore, it has been speculated that ET-B receptor mediated efferent arteriole vasoconstriction is only evident at higher concentrations because of relatively low affinity ligand binding (381). Such concentrations might be expected in the activated ET-1 system of portal hypertension.

In healthy humans, ET-1 infusion resulting in an increase in renal vascular resistance and fall in total renal blood flow was associated with a 36% increase in EFF (383). Moreover, when co-infused with an ET-A receptor antagonist at a dose that abolished the rise in renal vascular resistance exogenous ET-1 remained associated with a greater EFF relative to baseline. This supports the concept of ET-1 playing a role in preserving glomerular perfusion pressure, either via the ET-B receptor or indirectly via the action of other vasoactive mediators (384). In patients with CKD acute ET-A, but not combined ET-1, receptor antagonism caused renal vasodilatation and a fall in EFF while ET-B blockade did the opposite (315). Equally, long standing ET-A receptor antagonism in proteinuric CKD did not affect total renal blood flow but was associated with a lower EFF at the end of treatment (340). In this group, therefore, there is also evidence of preferential efferent arteriole vasoconstriction, although this may be mediated by the ET-A receptor.

In portal hypertension the apparent importance of increased tone in the efferent arteriole for maintaining glomerular perfusion pressure is echoed in studies of angiotensin II receptor blockade. The administration of captopril to patients with cirrhosis has had variable effects on total renal blood flow, but has consistently been found to reduce the EFF (385,386,387). Of potential relevance is that the RAAS and ET-1 systems are known to interact and haemodynamic synergism has been demonstrated in health (325,388). Only in the presence of angiotensin-converting enzyme inhibition did ET-A receptor antagonism cause renal vasodilatation and reduced EFF (325). Therefore, ET-1 and angiotensin II may act synergistically on the efferent arteriole (325). In CKD these vascular effects may promote proteinuria and

worsen renal outcomes (340). Conversely, in portal hypertension-related renal dysfunction they may be beneficial and serve to maintain glomerular perfusion pressure and GFR. Interestingly, cirrhotic patients with ascites who have a reduced GFR in the setting of preserved renal plasma flow but reduced EFR have evidence of inappropriately low systemic generation of angiotensin II (90). Relative under-activity of the RAAS may thus also be relevant to our findings.

The most striking observation of this study was the dramatic fall in UFR with ET-1 antagonism that was disproportionate to the fall in GFR. The effect was dose-dependent and only statistically significant following combined ET-A and ET-B receptor blockade. This is consistent with in vitro and animal models that provide strong evidence that ET-1 via the ET-B receptor is an important inhibitor of collecting duct sodium and water reabsorption (322,333,334,335,336). To our knowledge this is the first study in healthy humans or patients to demonstrate renal tubular effects of ET-1 receptor blockade in the absence of angiotensin-converting enzyme inhibition. ET-A receptor antagonism in healthy humans pretreated with enalapril increased sodium excretion markedly (325).

In contrast to in health, CKD and less advanced cirrhosis, we did not demonstrate any systemic haemodynamic effects of selective or combined ET-1 blockade (315,359). Although this may reflect small patient numbers no clear trends were seen. Furthermore, the lack of circulatory changes echoes the findings in Wong's non placebo controlled study of combined ET-1 receptor antagonism in patients with advanced liver disease (372). An alternative explanation is the competing effects of

the ET-A and ET-B receptors, particularly if ET-B mediated vasoconstriction has a greater role. This may also underpin our failure to demonstrate any dose-related renal haemodynamic changes.

An additional interesting observation that should be mentioned is the renal haemodynamic changes of the placebo arm. Patients demonstrated a dramatic increase in ERVR from baseline to study end, and fall in ERBF. GFR was maintained in the face of an increase in EFF. The immediate effect of a 500ml bolus of dextran 40 in cirrhotic patients with ascites is a rise in cardiac output and renal blood flow, and fall in renal resistance (454). Our 'baseline' measurements were performed 3 hours following a 600ml 5% dextrose bolus, with 250ml per hour maintenance thereafter. Dextrose is not an effective plasma expander and is rapidly lost from the intravascular compartment (455). Therefore, it seems possible that the rise in ERVR reflects a homeostatic response to fluid redistribution, with increased activity of the RAAS and SNS, and more subtle systemic haemodynamic changes that could not be identified with the methodology. Unfortunately readings were not taken prior to volume filling, but we speculate that the lower ERBF and higher ERVR may be closer to the normal resting values for these individuals.

We examined the effects of ET-1 receptor antagonism in patients with refractory ascites. Only 1 patient fulfilled the diagnostic criteria for type 2 hepatorenal syndrome and it could be argued that our results cannot be extrapolated to this group specifically (105). However, portal hypertension-related renal dysfunction should be considered as a spectrum (4). Moreover, the limitations of serum creatinine as a

marker of renal function in cirrhosis are well recognised (389). All 4 patients had a reduced GFR and half had a GFR of less than 60 ml/min/1.73m² (389). Despite relatively well preserved total renal blood flow the ERF was reduced and comparable to patients with hepatorenal syndrome (90). Therefore, the recruited patients had severe portal-hypertension-related renal dysfunction at time of inclusion characterised by failure of intra-renal compensatory mechanisms. Our findings are also similar to the renal effects demonstrated when Wong administered a non selective ET-1 receptor antagonist to patients with type 2 hepatorenal syndrome (372).

The main other limitation of this study is the small number of patients recruited. Nevertheless, we have demonstrated statistically significant effects of ET-1 receptor blockade in accordance with previous results. Furthermore, the principle factor limiting the number of potential participants was the numerous exclusion criteria. This has allowed an uncontaminated examination of ET-1 receptor antagonism in portal hypertension and refractory ascites to be performed. The small patient numbers is compounded by the wide swings in UFR demonstrated by patient 2 necessitating her exclusion from the analysis. A peak change of 700% from baseline is difficult to explain physiologically. However, all patients had a urinary catheter and careful attention was paid throughout the studies to ensure good drainage. The baseline parameters of patient 2 were somewhat different from the other participants with a lower SVRI and higher cardiac index, higher GFR and lower UFR. In retrospect, she may have had an element of superimposed acute alcoholic hepatitis, which could possibly alter the haemodynamic and renal response to ET-1.

2.6 Conclusion

In conclusion, in this double-blind placebo-controlled crossover study we have demonstrated for the first time that in patients with advanced cirrhosis and refractory ascites acute combined ET-A and ET-B receptor blockade caused a fall in GFR despite no change in systemic haemodynamics or total renal blood flow, and a marked reduction in UFR. Selective ET-A receptor antagonism had no haemodynamic or renal tubular effects suggesting that the ET-B receptor plays a key role in this setting. These findings are consistent with a reno-protective role for ET-1 in portal hypertension-related renal dysfunction.

CHAPTER 3

Renal dysfunction in acute liver failure: The systemic inflammatory response syndrome and its association with acute kidney injury in patients with acute liver failure

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3.1 Introduction

Renal failure is a common complication of ALF that occurs in 43% of patients with grade IV hepatic encephalopathy (163). It is associated with increased mortality, emphasised by the inclusion of serum creatinine in the KCH prognostic criteria (164,165). Despite the clinical burden of renal dysfunction in this setting, the aetiological mechanisms and risk factors remain unclear. Consequently, treatment options without liver transplantation are limited and potential prophylactic measures are unknown.

Current hypothesis suggests that the renal dysfunction of ALF and hepatorenal syndrome of cirrhosis share similar pathophysiology (105,169). Supporting this argument, the circulatory dysfunction of ALF, which is characterised by reduced systemic vascular resistance and high cardiac output, appears to parallel that of CLD (170,171,172,184). The marked reduction in renal blood flow and GFR that is associated with renal failure mirrors the renal perfusion changes of advanced cirrhosis (171). However, ALF has distinct haemodynamic features that indicate that the renal dysfunction of ALF and cirrhosis may not be comparable. Firstly, clinically significant portal hypertension is not always present in patients with ALF and renal dysfunction (172). Moreover, the degree of portal hypertension rarely equates with that of hepatorenal syndrome in cirrhosis: the mean reported hepatic venous pressure gradient for patients with ALF and renal dysfunction is 14 mmHg compared with 21 mmHg for patients with hepatorenal syndrome (109,148,172). Secondly, animal studies imply that the systemic vasodilatation may be more generalised and not

limited to the splanchnic circulation as occurs in cirrhosis (198,199). Pigs with ALF demonstrate reduced hind leg and renal vascular resistance, which contrasts with the femoral and renal vasoconstriction of hepatorenal syndrome (11,12,198,199). Vasodilatation within these vascular beds is more in keeping with the hyperdynamic syndrome of sepsis than of advanced cirrhosis (182,183). Recently it had been demonstrated that the SIRS is often present in patients with ALF (175,176). SIRS is associated with progression of hepatic encephalopathy suggesting that the systemic inflammatory response may be involved in its pathogenesis (175,176). Following on from this, we postulate that the systemic inflammatory response may play a role in the pathogenesis of renal dysfunction in these patients.

Additional factors that may contribute to renal dysfunction in ALF include hypovolaemia, nephrotoxic drugs, infection and disseminated intravascular coagulation (163,206,207,208). Paracetamol has been shown in animal models to have a direct nephrotoxic effect and in humans there are case reports of renal failure following paracetamol overdose in the absence significant hepatic injury (209,210,211). Nevertheless, the frequency of renal dysfunction in patients with paracetamol-induced ALF has not been shown to be higher than in other aetiologies (163).

3.2 Aims

At present there is a poor understanding of the pathogenesis and risk factors for renal dysfunction in ALF. The primary aim of this study was to examine whether SIRS is associated with renal dysfunction in a large cohort of patients with ALF. The secondary aim was to identify additional risk factors for the development of renal dysfunction in ALF.

3.3 Methods

This was a retrospective study of 442 patients admitted to a single tertiary referral centre with ALF between November 1992 and June 2007. One hundred and seven patients who were ventilated prior to admission were excluded from the analysis because of the influence of sedation and mechanical ventilation on SIRS (175,176). A further 6 patients who received RRT but did not fulfil the definition for renal dysfunction, and 21 patients who did not have a peak serum creatinine available, were also not assessed. Therefore, the study cohort comprised 308 patients.

ALF was defined as severe liver injury with hepatic encephalopathy in which the onset of encephalopathy was within 8 weeks of the first symptoms of illness, and in the absence of pre-existing liver disease (159). A patient was considered to have significant renal dysfunction if they fulfilled the RIFLE criteria (acronym indicating Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal disease) for AKI: peak serum creatinine ≥ 2 times the baseline level (390). The baseline serum creatinine was unavailable and therefore estimated from the Modification of Diet in Renal Disease (MDRD) Study 4-variable equation

$$\text{eGFR} = 186 \times \text{creatinine}(\text{mg/dl})^{-1.154} \times \text{age}(\text{years})^{-0.203} \times 1.212(\text{if black}) \times 0.742(\text{if female})$$

with an assumed GFR at the lower end of normal (75 ml/min/1.73m²), as outlined by the Acute Dialysis Quality Initiative (ADQI) workgroup (390). Serum creatinine may theoretically overestimate GFR in patients with ALF. Therefore, we chose the criteria for AKI rather than Acute Kidney Failure to allow detection of significant renal dysfunction with greater sensitivity (390).

Data was collected prospectively and entered into a dedicated database. The following variables were recorded at the time of hospital admission; temperature, pulse, white cell count (WCC), neutrophil count, platelet count, international normalised ratio (INR), serum electrolytes, serum bilirubin, alanine aminotransferase (ALT), albumin, and arterial hydrogen ion (H⁺), bicarbonate (HCO₃⁻), PaCO₂, and lactate. Peak creatinine and peak INR were documented. SIRS was defined as 2 or more of temperature <36°C or >38°C, heart rate >90 beats per minute, WCC <4×10⁹/L or >12×10⁹/L, and PaCO₂ <4.3 kPa (212). Regular alcohol intake prior to admission was recorded; alcohol excess was defined for women as >112 grams/week and for men >168 grams/week as per the UK guidelines (391). The presence of the following variables at any point during admission were documented; ventilation, treatment for increased intra-cranial pressure (ICP), hypotension (systolic blood pressure <90mmHg), need for inotropes (nor-adrenaline/adrenaline), hypoglycaemia, prognosis assessed using the KCH criteria, and infection (positive cultures and/or ascitic fluid polymorphonuclear count >250/mm³ and/or radiological evidence of infection) (165). All patients had cultures (sputum/stool/ascites/intra-vascular catheter where appropriate) and a chest X-ray performed routinely on admission, and repeat cultures if clinically indicated. Spontaneous survival and death were defined

as survival without liver transplantation and death without liver transplantation respectively: patients who received a liver transplant were excluded from all survival analyses.

Statistical analyses

Normally distributed continuous variables and non-parametric continuous variables were compared using the Student's t-test and Mann-Whitney test respectively. Chi-squared analysis was used for comparison of categorical data. Stepwise backwards logistic regression models, verified with forwards models, were used to determine the factors independently associated with death and AKI. Patients who received a liver transplant were excluded from all survival analyses. Only those variables with $P < 0.10$ were included in the multivariate analyses. $P < 0.05$ was considered significant. Data was analysed using the SPSS 15 package.

All values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

3.4 Results

Patient characteristics

The mean age was 39.7 (SD 14.7) years and the male to female ratio was 1:1.3. The causes of ALF were paracetamol overdose (217 patients), seronegative hepatitis (39 patients), idiosyncratic drug reaction (23 patients), hepatitis B (5 patients), autoimmune (5 patients), alcoholic hepatitis (5 patients), Budd-Chiari (5 patients), acute fatty liver of pregnancy (3 patients), hepatitis A (2 patients), Wilsons disease (2 patients) and non-paracetamol drug overdose (2 patients). One hundred and twelve patients died, 112 survived and 83 underwent liver transplantation.

Prevalence of acute kidney injury

At the time of admission to hospital the median serum creatinine was 149 (IQR 96-256) $\mu\text{mol/l}$, and 133 patients (43%) fulfilled the criteria for AKI. Thereafter, most patients demonstrated a decline in renal function: 196 patients (64%) had a 10% or greater increase in serum creatinine, 106 patients (34%) had no change in serum creatinine and 6 patients (2%) had a 10% or greater improvement in serum creatinine. The median peak serum creatinine was 288 (IQR 135-392) $\mu\text{mol/l}$. Two hundred and eight patients (67%) fulfilled the criteria for AKI at any point during their illness, of whom, 70% underwent RRT.

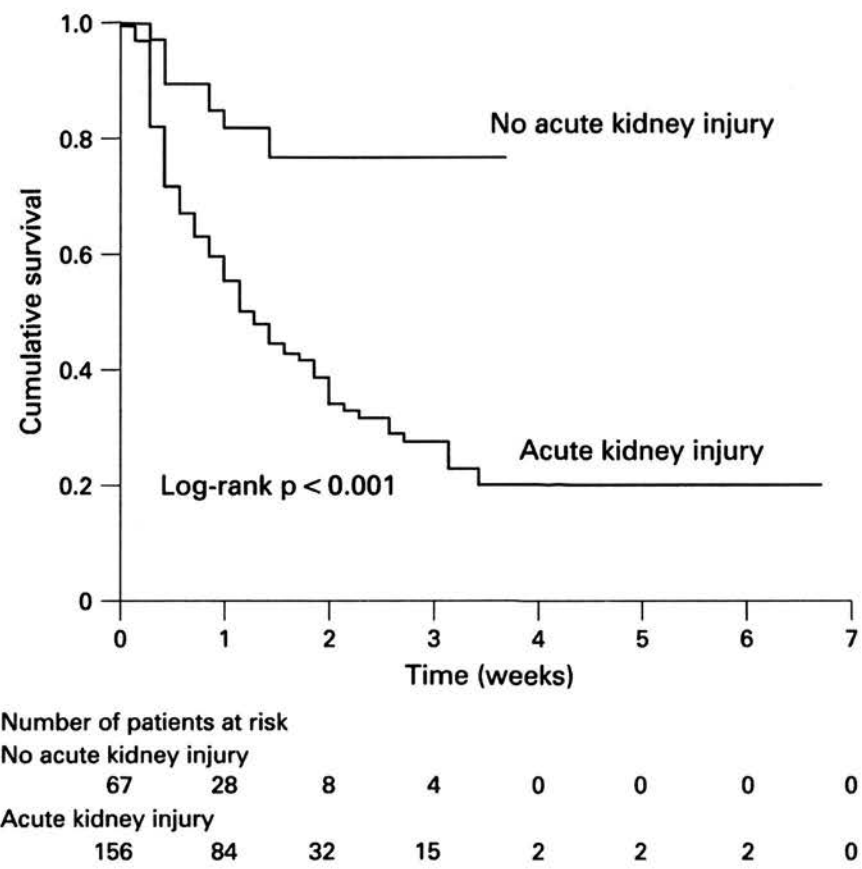
Prevalence of systemic inflammatory response syndrome

Seventy percent of patients had SIRS. SIRS was more prevalent in patients with paracetamol-induced ALF compared with patients with non-paracetamol-induced ALF (77% vs. 54%, $p<0.001$). The frequency of SIRS was not affected by the presence of infection (infected 70%: non-infected 70%, $p=0.880$). SIRS was not more common in patients who achieved KCH poor prognostic criteria (achieved 72%; not achieved 67%, $p=0.344$), although a greater proportion of patients who were hypoglycaemic (hypoglycaemic 78%, non-hypoglycaemic 65%, $p=0.040$) or required treatment for increased ICP (treatment 79%, no treatment 66%, $p=0.032$) demonstrated SIRS.

Acute kidney injury, systemic inflammatory response syndrome and mortality

Patients with AKI had a prolonged hospital admission (AKI 11;7-20 days: non-AKI 6;4-10 days, median and IQR, $p<0.001$) and reduced spontaneous survival (AKI 36%; non-AKI 84%, $p<0.001$, Figure 3.1). There was no relationship between AKI and liver transplantation (AKI 25%; non-AKI 33%, $p=0.118$). However, the transplanted patients who had AKI pre-operatively had a longer post-operative hospital stay (AKI 26;20-33 days: non-AKI 15;13-20 days, median and IQR range, $p<0.001$) and a trend towards reduced survival to hospital discharge (AKI 67%; non-AKI 85%, $p=0.064$).

Figure 3.1: Spontaneous survival in patients with ALF subdivided based on the presence or absence of AKI. Patients who received a liver transplant were excluded from the survival analysis.



Abbreviations: AKI, acute kidney injury; ALF, acute liver failure.

The presence of SIRS was also associated with reduced spontaneous survival (SIRS 42%; non-SIRS 60%, $p=0.035$). Nevertheless, a similar proportion of patients with and without SIRS received a liver transplant (26% vs. 32%, $p=0.386$) and there was no association between SIRS and survival following transplantation (SIRS 70%; non-SIRS 71%, $p=0.957$).

Other variables associated with spontaneous survival are outlined in Table 3.1. On multivariate analysis AKI, and not SIRS, was independently associated with mortality.

Table 3.1: Factors predictive of mortality on univariate and multivariate analysis in patients with ALF*.

	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Age (years)	1.04(1.02-1.06)	<0.001	1.06(1.03-1.09)	<0.001
Hypoglycaemia	6.20(3.40-11.27)	<0.001	4.97(2.27-10.91)	<0.001
Treatment for increased ICP	5.17(2.54-10.55)	<0.001	6.72(2.56-17.67)	<0.001
SIRS	2.00(1.04-3.85)	0.037		
AKI	9.18(4.45-18.94)	<0.001	5.48(2.20-13.64)	<0.001

Reference group (relative risk 1.00): no hypoglycaemia, no treatment for increased ICP, no SIRS, no acute kidney injury, male gender, non-paracetamol-induced ALF, no infection.

*Variables not associated with mortality: female gender (p=0.502), paracetamol-induced ALF (p=0.129), infection (p=0.547).

Abbreviations: ALF, acute liver failure; OR, odds ratio; CI, confidence interval; ICP, intra-cranial pressure; SIRS, systemic inflammatory response syndrome; AKI, acute kidney injury.

Relationship between acute kidney injury and the aetiology of acute liver failure

Patients who developed AKI were more likely to have ALF as a result of paracetamol ingestion than those who did not have AKI (80% vs. 51%, $p<0.001$). The median peak serum creatinine for patients with paracetamol-induced ALF was 300 (IQR 183-413) $\mu\text{mol/l}$ and for patients with non-paracetamol-induced ALF was 149 (IQR 99-322) $\mu\text{mol/l}$ ($p<0.001$). Seventy-six percent of patients with paracetamol-induced ALF and 46% of patients with non-paracetamol-induced ALF fulfilled the criteria for AKI ($p<0.001$).

Relationship between acute kidney injury and the severity of acute liver failure

Clinical and biochemical characteristics of the AKI and non-AKI patients on admission to hospital are outlined in table 3.2. Patients with AKI had a higher ALT level ($p<0.001$), INR ($p=0.001$) and lactate ($p=0.007$), and were more likely to be acidotic (31% vs. 0%, $p<0.001$).

By definition all patients became encephalopathic. However, the AKI group were more likely than the non-AKI group to be ventilated (77% vs. 56%, $p<0.001$) and a greater proportion required treatment for increased ICP (AKI 34%, non-AKI 14%, $p<0.001$). In addition, patients with AKI were more likely to demonstrate hypoglycaemia (AKI 50%, non-AKI 18%, $p<0.001$) and to fulfil KCH poor prognostic criteria (AKI 64%, non-AKI 37%, $p<0.001$).

Table 3.2: Clinical and biochemical characteristics on admission of patients with ALF who did/did not develop AKI.

Variable	AKI (no:208)	Non-AKI (no:100)	p value
Age (years)	41.0+/-14.0	36.9+/-15.7	0.020
Male:Female	1:1.2	1:1.4	0.439
Alcohol excess (%)	88(52)	30(38)	0.043
Temperature (°C)	36.4+/-1.1	36.8+/-0.8	0.001
Heart rate (bpm)	107(92-120)	96(80-110)	<0.001
WCC (x10 ⁹ /l)	12.8(9.4-18.0)	11.0(6.9-13.7)	0.001
Neutrophil count (x10 ⁹ /l)	11.2 (7.6-16.5)	8.8(5.9-12.2)	<0.001
Lymphocyte count (x10 ⁹ /l)	0.7(0.5-1.2)	1.0(0.7-1.5)	0.002
Platelet count (x10 ⁹ /l)	115(55-162)	158(99-237)	<0.001
INR	4.9(3.0-7.1)	3.7(2.5-5.6)	0.001
Sodium (mmol/l)	134(129-138)	136(133-138)	0.007
Potassium (mmol/l)	4.1(3.6-5.0)	3.6(3.3-4.2)	<0.001
Urea (mmol/l)	7.7(5.0-12.5)	4.1(2.7-7.4)	<0.001
Creatinine (µmol/l)	221(138-298)	92(74-113)	<0.001
Bil (µmol/l)	96(68-139)	140(88-433)	<0.001
ALT (U/l)	5856(2105-10000)	2655(957-6775)	<0.001
Albumin (g/l)	32.9+/-8.2	32.9+/-7.2	0.946
H+ (nmol/l)	41(35-50)	33(31-37)	<0.001
PaCO ₂ (kPa)	4.0(3.2-4.6)	4.2(3.8-4.8)	0.033
HCO ₃ ⁻ (mmol/l)	18.1 (13.7-22.8)	24.0(20.9-26.9)	<0.001
Lactate (mmol/l)	4.2(2.6-8.4)	2.7(2.0-4.5)	0.007
Any encephalopathy (%)	125(61)	68(68)	0.253
SIRS (%)	136(78)	43(53)	<0.001

Abbreviations: ALF, acute liver failure; AKI, acute kidney injury; WCC, white cell count; INR, international normalised ratio; ALT, alanine aminotransferase; SIRS, systemic inflammatory response syndrome.

Relationship between acute kidney injury and systemic inflammatory response syndrome

Patients who developed AKI had evidence of a greater systemic inflammatory response. At the time of hospital admission they were more likely to have a temperature $<36^{\circ}\text{C}$ (AKI 34%, non-AKI 12%, $p=0.010$), had a faster heart rate ($p<0.001$), a higher WCC ($p=0.001$) and a lower PaCO_2 ($p=0.033$) (table 3.2). Furthermore, a greater proportion of those with AKI developed hypotension (AKI 63%, non-AKI 13%, $p<0.001$) and required inotropic support (AKI 56%, non-AKI 9%, $p<0.001$).

Seventy-eight percent of the AKI patients had SIRS compared with 53% of the non-AKI patients ($p<0.001$). An increasing number of components of SIRS was associated with an increased probability of renal dysfunction: 47%, 60%, 69%, 79% and 81% of the patients with 0, 1, 2, 3 and 4 components of SIRS at admission respectively developed AKI ($p=0.047$). The AKI group were more likely to demonstrate infection (AKI 56%, non-AKI 38%, $p=0.003$). Nevertheless, in both those with infection (AKI 74%, non-AKI 57%, $p=0.062$) and those without infection (AKI 82%, non-AKI 50%, $p<0.001$) SIRS was more common in the AKI patients.

Independent risk factors for the development of acute kidney injury in acute liver failure

A multivariate analysis was performed to identify the factors that are independently associated with renal dysfunction (Table 3.3). This revealed that the severity of ALF, the systemic inflammatory response to ALF, and superimposed factors may all be relevant. The variables independently associated with AKI were age ($p=0.024$), fulfilled KCH poor prognostic criteria ($p<0.001$), hypotension ($p<0.001$), SIRS ($p=0.017$), superimposed infection ($p=0.077$) and paracetamol as the cause of ALF ($p<0.001$).

Table 3.3: Factors predictive of AKI on univariate and multivariate analysis in all patients with ALF.

Variable	Univariate OR (95% CI)	p value	Multivariate OR (95% CI)	p value
Age (years)	1.02(1.00-1.04)	0.021	1.03(1.00-1.06)	0.024
Paracetamol-induced ALF	3.80(2.26-6.38)	<0.001	10.72(4.24-27.09)	<0.001
KCH poor prognosis	3.08(1.88-5.06)	<0.001	6.33(2.65-15.13)	<0.001
Hypoglycaemia	4.44(2.49-7.94)	<0.001		
Treatment for increased ICP	3.03(1.60-5.73)	0.001		
Hypotension	11.23(5.87-21.48)	<0.001	7.01(3.06-16.07)	<0.001
SIRS	3.16(1.80-5.57)	<0.001	2.42(1.17-5.00)	0.017
Infection	2.07(1.27-3.39)	0.004	1.93(0.93-4.02)	0.077

Reference group (relative risk 1.00): non-paracetamol-induced ALF, did not achieve KCH prognostic criteria. No hypoglycaemia, no treatment for increased ICP, no hypotension, no SIRS, no infection.

Abbreviations: AKI, acute kidney injury; ALF, acute liver failure; OR, odds ratio; CI, confidence interval; ICP, intra-cranial pressure; SIRS, systemic inflammatory response syndrome.

In view of the strong association of paracetamol with AKI the entire cohort was then subdivided into two groups: paracetamol-induced ALF and non-paracetamol-induced ALF based on the presence or absence of paracetamol-induced liver injury respectively. The associations between the severity of ALF and AKI, and SIRS and AKI, were reassessed.

Acute kidney injury in paracetamol-induced acute liver failure

In the paracetamol-induced ALF subgroup, patients with AKI had evidence of more severe liver injury when compared with patients with no AKI (Table 3.4): they were more likely to be hypoglycaemic ($p<0.001$), to require treatment for increased ICP ($p=0.003$) and to fulfil KCH poor prognostic criteria ($p<0.001$). In contrast, the AKI patients were not more likely to demonstrate SIRS ($p=0.373$) and were not more likely to have infection ($p=0.287$). However, they did have a lower admission temperature ($p<0.001$), higher WCC ($p=0.043$), trend towards a lower PaCO₂ ($p=0.070$), and were more likely to have 3 or more systemic inflammatory response components (AKI 43%, non-AKI 27%, $p=0.055$). On multivariate analysis the factors associated with AKI in patients with paracetamol-induced ALF were age (OR 1.03; 95% CI 1.00-1.06, $p=0.059$), treatment for increased ICP (OR 2.74; 95% CI 1.05-7.15, $p=0.039$) and hypotension (OR 11.38; 95% CI 4.19-30.91, $p<0.001$).

Table 3.4: Univariate analysis of variables as predictors of AKI in patients with paracetamol-induced ALF and non-paracetamol-induced ALF.

Variable	Paracetamol-induced ALF		Non-paracetamol-induced ALF	
	AKI (no:166)	Non-AKI (no:51)	AKI (no:42)	Non-AKI (no:49)
Peak creatinine ($\mu\text{mol/l}$)	341(294-454)	109(86-140)	329(258-474)	99(86-113)
Renal replacement therapy (%)	124(75)	0	22(52)	0
Age (years)	39.5+/-13.1	33.6+/-14.2 ^b	47.4+/-15.5	40.3+/-16.6 ^a
Male:Female	1.1.0	1:1.2	1:2.0	1:1.7
Alcohol excess (%)	80(57)	22(54)		
Jaundice-enceph time (days)			11(2-25)	12(2-23)
Temperature OA ($^{\circ}\text{C}$)	36.4+/-1.1	37.0+/-0.9 ^c	36.3+/-1.3	36.6+/-0.6
Heart rate OA (bpm)	109(92-120)	102(86-120)	100(90-120)	90(80-100) ^b
WCC OA ($\times 10^9/\text{l}$)	13.0(9.4-18.0)	11.5(6.9-14.3) ^a	12.0(9.4-18.0)	10.7(7.0-12.8) ^a
Neutrophil count OA ($\times 10^9/\text{l}$)	11.5(8.2-16.5)	10.5(5.9-12.7)	9.6(7.0-14.9)	7.7(5.5-10.4) ^a
Platelet count OA($\times 10^9/\text{l}$)	112(47-157)	154(92-220) ^b	136(77-182)	164(113-284) ^a
INR OA	5.3(3.6-7.7)	4.6(3.1-6.6) ^a	2.4(1.9-4.3)	2.7(2.0-4.2)
Peak INR	7.9(5.0-10.7)	6.4(4.3-9.3)	4.3(2.2-5.1)	3.2(2.3-5.1)
Bilirubin OA ($\mu\text{mol/l}$)	89(62-116)	100(74-124)	325(110-531)	438(241-524)
ALT OA (U/l)	6949(3884-10925)	6547(4373-8790)	613(171-2712)	1050(365-2228)
Albumin OA (g/l)	34.3+/-8.1	36.5+/-6.1	28.1+/-6.7	29.2+/-6.4
H+ OA (nmol/l)	41(35-50)	33(30-38) ^c	41(34-45)	34(31-36) ^b
PaCO ₂ (kPa)	4.0(3.2-4.6)	4.2(3.7-4.8)	4.0(3.4-5.1)	4.4(3.8-4.7)
HCO ₃ ⁻ OA (mmol/l)	18.0(13.0-22.4)	23.0(20.0-26.9) ^c	20.3(15.1-24.7)	24.6(21.8-27.0) ^a
Lactate OA (mmol/l)	4.7(2.8-9.0)	3.0(2.1-5.3)	3.7(2.3-7.0)	2.5(1.7-4.0)
Hypotension (%)	102(63)	5(10) ^c	27(64)	8(17) ^c
Inotropes (%)	98(60)	4(8) ^c	18(43)	5(10) ^c
Hypoglycaemia (%)	89(55)	11(22) ^c	13(31)	7(15)
Treatment for increased ICP(%)	59(36)	7(14) ^b	10(24)	7(15)
Infection (%)	89(55)	23(46)	26(63)	15(31) ^b
SIRS (%)	110(79)	28(72)	26(76)	15(36) ^c
KCH poor prognosis (%)	105(63)	0(0) ^c	29(69)	37(76)
Spontaneous survival (%)	48(37)	47(92) ^c	8(30)	10(63) ^a

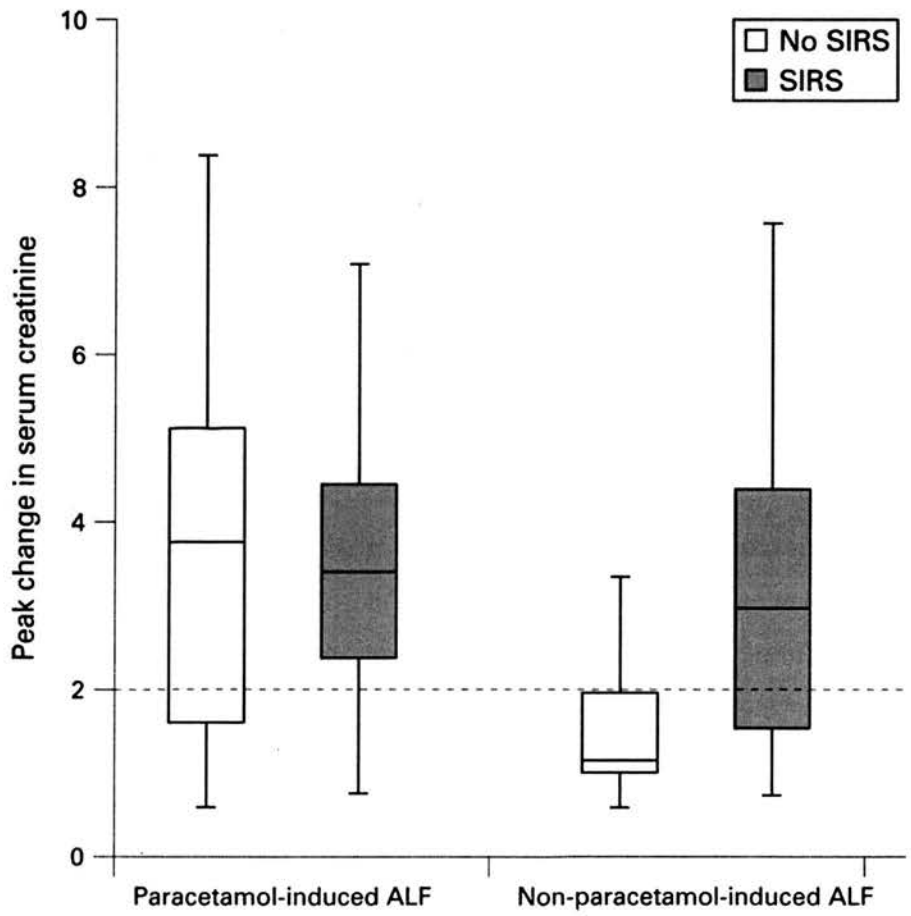
^a p<0.05, ^b p<0.01, ^c p<0.001 versus no acute kidney injury group

Abbreviations: AKI, acute kidney injury; ALF, acute liver failure; OA, on admission to hospital; WCC, white cell count; INR, international normalised ratio; ALT, alanine aminotransferase; ICP, intra-cranial pressure; SIRS, systemic inflammatory response syndrome; KCH, Kings College Hospital.

Acute kidney injury in non-paracetamol-induced acute liver failure

In the non-paracetamol-induced ALF subgroup, patients with AKI did not have evidence of more severe liver injury relative to patients with no AKI (table 3.4). Despite a trend towards an association between AKI and hypoglycaemia ($p=0.062$), patients with renal dysfunction were not more likely to require treatment for increased ICP ($p=0.285$) or to achieve KCH poor prognostic criteria ($p=0.491$). Nevertheless, there was a strong relationship between AKI and SIRS ($p<0.001$, Figure 3.2). Patients with AKI were more likely to have infection ($p=0.002$), and in both infected (AKI 78%, non-AKI 40%, $p=0.017$) and non-infected patients (AKI 73%, non-AKI 33%, $p=0.037$) the prevalence of SIRS was greater in those with AKI. On multivariate analysis the factors associated with AKI in patients with non-paracetamol-induced ALF were hypotension (OR 8.63; 95% CI 2.63-28.3, $p<0.001$) and SIRS (OR 6.98; 95% CI 2.13-22.83, $p=0.001$).

Figure 3.2: Boxplot of peak change in serum creatinine (peak serum creatinine/baseline serum creatinine) in patients with ALF subdivided based on aetiology, and the presence or absence of the SIRS. A peak change in serum creatinine ≥ 2 , represented by the horizontal line, is the definition of AKI.



Abbreviations: AKI, acute kidney injury; ALF, acute liver failure; SIRS, systemic inflammatory response syndrome.

3.5 Discussion

We have shown for the first time that in ALF renal dysfunction is associated with SIRS. On univariate analysis patients with AKI were more likely to be hypothermic, had a faster heart rate, a higher WCC and a lower PaCO₂. Multivariate analysis confirmed that SIRS was a risk factor for the development of AKI. Importantly, this association was independent of the presence of infection and of severity of liver injury as assessed by the KCH prognostic criteria. A further novel finding of our study is the strikingly increased risk of AKI demonstrated by patients with paracetamol-induced ALF. This relationship supports in vitro animal data and clinical suspicion that paracetamol has a direct nephrotoxic effect. Drug induced renal injury could mask any relationship between SIRS and renal dysfunction. Therefore, a key finding of our study is the strong association between SIRS and AKI in the subgroup of patients with non-paracetamol-induced ALF.

To our knowledge this is the first study to examine the prevalence of SIRS in ALF using all 4 components of the systemic inflammatory response (212). Previous groups have excluded the respiratory component because of the influence of mechanical ventilation (175,176,177). Using similar methodology to the earlier studies the prevalence of SIRS in our cohort was comparable (50%: Rolando *et al* 57%: Vaquero *et al* 34%: Schmidt *et al* 55%). However, when all 4 components were applied the prevalence of SIRS rose to 70%. The diagnosis of SIRS was based on parameters at time of admission to our unit. Therefore, additional patients may have developed SIRS at a later stage.

The observation that SIRS may be present in patients with ALF even in the absence of infection confirms previous findings (175,176). In fact, the prevalence of SIRS was similar in infected and non-infected groups. We acknowledge that occult infection may have influenced our results. However, our unit actively seeks infection with cultures performed routinely on admission, and repeated thereafter if clinically indicated. SIRS is the clinical sequelae of a massive inflammatory cascade that results from systemic cytokine release (179). SIRS not only occurs in infected patients but also in a variety of other conditions including trauma and acute pancreatitis (179). In ALF, non-infected patients similarly demonstrate high circulating levels of cytokines (216,219,220,392). The source of the systemic cytokines in this setting remains unclear although release from the necrotic liver, or secondary to endotoxaemia, or impaired hepatic cytokine metabolism are possible (179,205,239).

The findings of our study suggest that the systemic inflammatory response plays a role in the pathogenesis of renal dysfunction in ALF. Consequently, the renal dysfunction of sepsis may be a more accurate parallel than the hepatorenal syndrome of cirrhosis. In sepsis renal dysfunction probably occurs as a result of haemodynamic and non-haemodynamic factors (183). There is reduced glomerular filtration pressure, which reflects altered intra- and extra-renal vascular activity (183,206). Furthermore, the systemic inflammatory response may contribute directly to renal tubular dysfunction by stimulating apoptotic death of tubular cells (183,393). Most research has focused on TNF- α . This cytokine appears to play a major role in the pathogenesis of the circulatory dysfunction and renal injury of sepsis (183,206,394).

In ALF TNF- α and IL-6 levels have been shown to correlate with the development of renal failure and circulatory failure respectively (395). Therefore, we propose that in ALF, as in sepsis, cytokines responsible for the systemic inflammatory response are central to the development of renal dysfunction.

The systemic inflammatory response has also been implicated in the pathogenesis of the circulatory and renal dysfunction of cirrhosis (143,396). These patients demonstrate endotoxaemia in the absence of infection and have increased systemic cytokine levels, which correlate with the severity of disease (32,116,117,119,397,398). Furthermore, prophylactic antibiotics reduce the incidence of hepatorenal syndrome and improve survival in patients with decompensated cirrhosis, independent of the prevention of infection (123). Nevertheless, portal hypertension remains the primary event (105,399). Moreover, the frequency of SIRS in patients with cirrhosis, even when renal dysfunction is present (41%), is much less than we have demonstrated in ALF (AKI 78%, non-AKI 53%) (396). Patients with subfulminant ALF are more likely to have clinically significant portal hypertension than those with fulminant ALF, and may develop ascites (172). Therefore, in subfulminant ALF renal dysfunction may share similarities with hepatorenal syndrome of cirrhosis. However, in fulminant hepatic failure the systemic inflammatory response may be the key mediator of renal dysfunction.

We were unable to demonstrate a relationship between the severity of ALF, as assessed by the KCH prognostic criteria, and SIRS. It is well recognised that there

are significant inter-individual differences in the systemic inflammatory response to infection and other forms of injury, which may be genetically predetermined (179,180). Therefore, it is likely that some individuals will be more at risk of the circulatory and renal complications of the systemic inflammatory response to ALF than others.

Paracetamol as the cause of ALF was an independent predictor of AKI consistent with the clinical suspicion that paracetamol has a direct nephrotoxic effect. The mode of paracetamol related nephrotoxicity remains undefined, although case reports of isolated renal failure following paracetamol overdose indicate that it is independent of hepatic injury (209,211). It is postulated that a locally produced toxic metabolite of paracetamol induces proximal tubular cell necrosis (209,210,400). Functional renal effects may also contribute as alterations in renal plasma flow and GFR have been demonstrated in the absence of structural and hepatic dysfunction (210).

Our study has some potential limitations. We chose to use Trey and Davidson's definition of ALF and include all patients with encephalopathy in the study. Grade I encephalopathy is difficult to diagnose and previous similar papers have included patients with severe encephalopathy only (163,164,171). However, our cohort was comparable to the described populations with regards to severity of liver injury and outcome, and the diagnosis of encephalopathy was made by a small number of physicians indicating relative consistency of opinion. Secondly, we did not have details of co-morbidities and, in particular, pre-existing renal disease or nephrotoxic medications that may have influenced our results. The patients studied were of a

young age and it is therefore assumed that pre-existing renal function was normal. Our unit avoids nephrotoxic drugs when managing patients with ALF although this does not preclude exposure prior to transfer.

The identification of modifiable risk factors for the development of AKI in ALF has important implications for patient management. As in sepsis, early and aggressive optimisation of haemodynamics with fluid therapy, central venous pressure monitoring and inotrope administration may significantly reduce the occurrence of renal dysfunction (206,401,402). Furthermore, manipulation of the systemic inflammatory response itself is likely to be advantageous. Prompt diagnosis and treatment of superimposed infection is essential (401,402). In addition, removal of cytokines and other inflammatory mediators by haemofiltration or albumin dialysis (MARS) may be beneficial (403,404,405). Finally, pentoxifylline, by down regulation of pro-inflammatory cytokines, is a possible future therapeutic option (406,407).

3.6 Conclusion

In conclusion, in this large single-centre retrospective study we have examined the relationship between SIRS and AKI in ALF. We have shown that the SIRS is strongly associated with renal dysfunction and we hypothesise that the inflammatory cascade plays a key role in its pathogenesis. Given the reduced spontaneous survival of patients with AKI we suggest that the early administration of therapies that target the systemic inflammatory response and limit the development of AKI may have a favourable effect on patient morbidity and mortality.

CHAPTER 4

Renal dysfunction as a prognostic indicator: Estimated glomerular filtration rate (eGFR) as a prognostic indicator in patients listed for liver transplantation

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4.1 Introduction

Cirrhosis is associated with a progressive functional renal impairment characterised by increased tubular sodium reabsorption, impaired free water clearance and pre-renal azotemia (4). This spectrum of renal dysfunction evolves in parallel with advancing disease and consequently the clinical manifestations of renal dysfunction, ascites, hyponatraemia and hepatorenal syndrome, are important prognostic markers (80,105,299,408). Serum creatinine as a continuous variable is an independent predictor of mortality following the transjugular intrahepatic portosystemic shunt procedure and in those on the liver transplant waiting list (409,410). It is a component of the MELD score, which is used to prioritise graft allocation.

However, serum creatinine is not solely influenced by glomerular filtration and is not an accurate estimator of renal function (411). Creatinine production is proportional to muscle mass, and is greater in males than females, in younger than older individuals and in blacks than whites, despite similar GFR (282). In addition, in cirrhosis reduced creatine production by the liver, muscle wasting and increased renal tubular secretion of creatinine may result in a falsely low serum creatinine level (412,413).

The effect of gender, age and race on serum creatinine is of particular concern in the MELD era of organ allocation. UNOS data has demonstrated that women listed for liver transplantation are less likely to survive to transplantation than men, supporting a systematic bias of the scoring system (414,415,416). Similarly, an inherent discrimination against older patients could explain the independent association of

increasing age with waiting-list mortality (417). It follows that a scoring system with an alternative measure of renal function may be preferable to MELD.

The gold standard measure of GFR, inulin clearance, has recently been shown to be superior to serum creatinine in predicting liver transplant waiting-list mortality (418). Unfortunately, inulin clearance is time consuming, impractical and costly and is not a useful test if repeated measures are required (412,413). Calculated GFR is a possible alternative and has been evaluated as an absolute measure of renal function, although not as a prognostic marker, in this setting (419).

The most accurate calculated GFR for cirrhotic patients is provided by the MDRD equations, which are creatinine based estimates modified for age, gender and race (419,420,421). The MDRD 4-variable calculated GFR is readily available, at minimal cost, with routine reporting advocated in several countries, and is an attractive measure of renal function (422,423). The MDRD 5-variable and 6-variable calculated GFRs, in addition, adjust for blood urea nitrogen, and blood urea nitrogen and serum albumin, respectively, and could be superior prognostic indicators.

4.2 Aims

The aim of this study was to examine whether the MDRD calculated GFR is superior to serum creatinine in predicting prognosis on the liver transplant waiting list. In a subgroup of patients measured creatinine clearance was also available and was examined as a prognostic indicator.

4.3 Methods

This was a single-centre retrospective study of consecutive adults listed for first liver transplantation between November 1992 and June 2007. Patients listed for ALF, hepatocellular carcinoma, or joint liver/kidney transplantation, or who had documented intrinsic renal disease were not assessed. Those removed from or still active on the waiting list were also not included.

The following variables at time of liver transplant assessment were recorded: gender, age, race, aetiology of liver disease, presence of ascites or hepatic encephalopathy, and laboratory data (serum sodium, creatinine, bilirubin, albumin and INR). eGFR was calculated from the relevant parameters using the MDRD study 4-variable (eGFR(MDRD4)), 5-variable (eGFR(MDRD5)) and 6-variable (eGFR(MDRD6)) equations (420,421).

$$\text{eGFR(MDRD4)} = 186 \times \text{creatinine(mg/dl)}^{-1.154} \times \text{age(ys)}^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$$

$$\text{eGFR(MDRD5)} = 270 \times \text{creatinine(mg/dl)}^{-1.007} \times \text{age(ys)}^{-0.180} \times \text{blood urea nitrogen(mg/dl)}^{-0.169} \times (0.755 \text{ if female}) \times (1.178 \text{ if black})$$

$$\text{eGFR(MDRD6)} = 170 \times \text{creatinine(mg/dl)}^{-0.999} \times \text{age(ys)}^{-0.176} \times \text{blood urea nitrogen(mg/dl)}^{-0.170} \times \text{albumin(g/dl)}^{+0.318} \times (0.762 \text{ if female}) \times (1.180 \text{ if black})$$

The MELD score was determined as previously described (424). The UKELD, a recently devised scoring system that incorporates serum sodium in addition to the MELD variables, was also calculated (425).

In a subgroup of patients transplanted between May 2000 and June 2007 measured creatinine clearance (CrCl) was available. This was determined from a 24-hour urinary collection performed routinely during the in-patient assessment period. Failure to obtain a CrCl was, in most cases, secondary to poor patient compliance.

Statistical analyses

Normally distributed continuous variables and non-parametric continuous variables of males and females were compared using the Student's t-test and Mann-Whitney test respectively. Chi-squared analysis was used for the comparison of categorical variables. Survival modelling was performed using cox proportional hazards regression. Data was censored at the time of liver transplantation and to lessen the influence of extreme values all continuous laboratory variables were transformed into their natural logarithms. To allow the comparison of MELD or UKELD with a similar model with logeGFR or logeCrCl substituted for logecreatinine the regression coefficients of MELD or UKELD were initially adjusted for our patient population. Regression coefficients were then recalculated in the presence of logeGFR instead of logecreatinine. Receiver-operating characteristic (ROC) curves were generated to assess the accuracy of models in predicting 3-month waiting-list mortality. Concordance statistics were compared using the method described by Hanley and

McNeil (426). All patients censored prior to the specified time point were excluded from these analyses. $p < 0.05$ was considered statistically significant at all times. Data was analysed using the SPSS 15 package.

Values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

4.4 Results

Patient characteristics

The mean age of the patients (number 427) at time of listing for liver transplantation was 55.3 (SD 11.6) years and the male to female ratio was 1:1. The main indications for transplantation were primary biliary cirrhosis (119 patients, 27.9%), alcoholic liver disease (103 patients, 24.1%), sclerosing cholangitis (62 patients, 14.5%), hepatitis C cirrhosis (37 patients, 8.9%) cryptogenic cirrhosis (36 patients, 8.4%), and autoimmune hepatitis (33 patients, 7.7%). The median listing MELD score was 16 (IQR 13-20) and the median listing UKELD score was 56 (IQR 54-60).

Sixty patients (14.1%) died prior to liver transplantation. The median time from listing to death was 50 (IQR 26-101) days. For patients who were transplanted the median waiting-time was 68 (IQR 27-142) days. Two hundred and twelve patients (49.6%) were transplanted and 44 patients (10.3%) died within 3 months of listing.

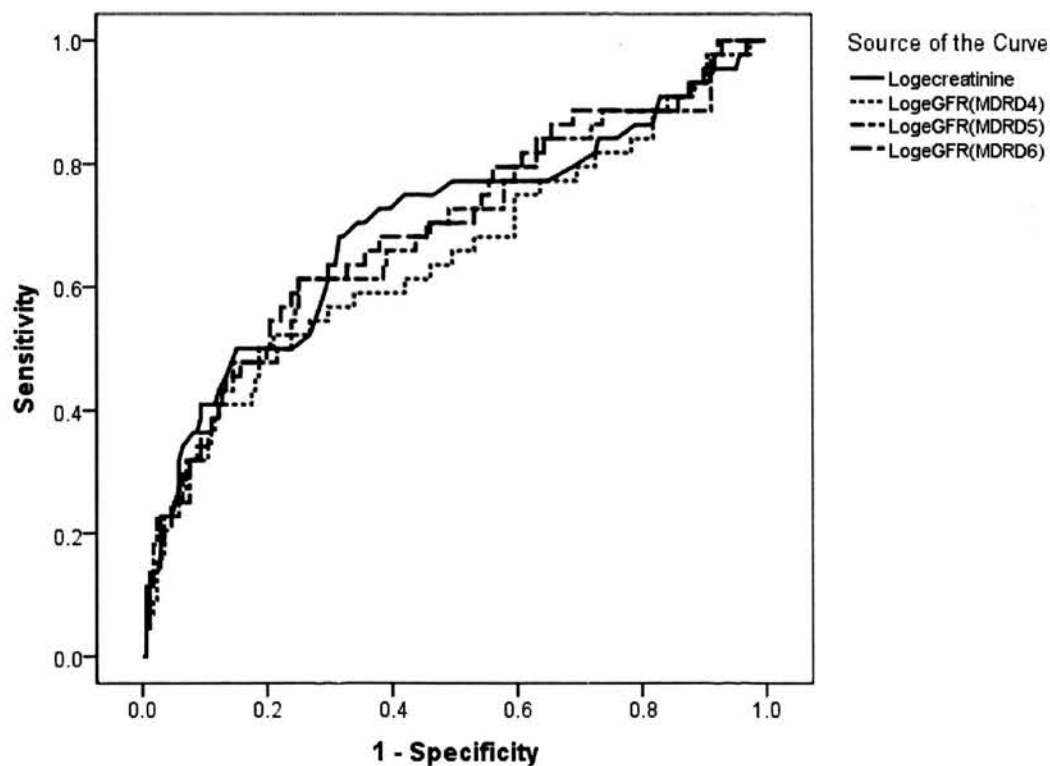
The median listing serum creatinine was 89 (IQR 77-107) $\mu\text{mol/l}$, the median listing serum sodium was 136 (IQR 132-139) mmol/l , and 60.6% of patients had ascites. The median eGFR(MDRD4), eGFR(MDRD5) and eGFR(MDRD6) was 69 (IQR 57-83) ml/min/1.73m^2 , 71 (IQR 56-86) ml/min/1.73m^2 , and 73 (IQR 57-89) ml/min/1.73m^2 , respectively.

Comparison of MDRD eGFR equations as predictors of waiting list mortality

Loge creatinine (OR 14.12; 95% CI 3.76-53.13, $p < 0.001$), logeGFR(MDRD4) (OR 0.18; 95% CI 0.06-0.53, $p = 0.002$), logeGFR(MDRD5) (OR 0.16; 95% CI 0.06-0.44, $p < 0.001$), and logeGFR(MDRD6) (OR 0.14; 95% CI 0.05-0.39, $p < 0.001$) demonstrated an association with 3-month waiting-list mortality.

ROC curves for loge creatinine, logeGFR(MDRD4), logeGFR(MDRD5) and logeGFR(MDRD6) as predictors of 3-month waiting list mortality are shown in Figure 4.1. When all eGFR equations were compared logeGFR(MDRD6) had the greatest concordance statistic (logeGFR(MDRD4) 0.648; 0.548-0.749; logeGFR(MDRD5) 0.683; 0.587-0.780; logeGFR(MDRD6) 0.695; 0.601-0.789, loge creatinine 0.696; 0.598-0.793, c-statistic and 95% confidence interval). LogeGFR(MDRD6) statistically outperformed logeGFR(MDRD4) ($p = 0.054$), and was comparable to logeGFR(MDRD5) ($p = 0.614$) and loge creatinine ($p = 0.981$). Following on from this, all further analyses comparing eGFR with serum creatinine were performed using the eGFR MDRD 6-variable equation.

Figure 4.1: ROC curves of log serum creatinine (logecreatinine), log eGFR calculated using the MDRD 4-variable equation (logeGFR(MDRD4)), log eGFR calculated using the MDRD 5-variable equation (logeGFR(MDRD5)) and log eGFR calculated using the MDRD 6-variable equation (logeGFR(MDRD6)) for predicting 3-month liver transplant waiting list mortality.



Abbreviations: eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; ROC, receiver-operating characteristic.

Does substitution of eGFR(MDRD6) for serum creatinine improve the prognostic accuracy of MELD and UKELD?

ROC analysis was used to determine whether the substitution of logeGFR(MDRD6) for logecreatinine improved the accuracy of the existing prognostic models, MELD and UKELD (Table 4.1). The regression coefficients for each model were initially adjusted for our study population (MELD(adj)/UKELD(adj)), and thereafter recalculated in the presence of logeGFR(MDRD6) instead of logecreatinine (MELD(eGFR)/UKELD(eGFR)).

Table 4.1: AUC for ROC curves for prediction of 3-month liver transplant waiting-list mortality in all patients.

Model	c-statistic	95% CI
MELD (adj)	0.841	0.773-0.909
MELD (eGFR)	0.846	0.777-0.915
UKELD (adj)	0.859	0.790-0.928
UKELD (eGFR)	0.864	0.795-0.933

Abbreviations: CI, confidence interval; c-statistic, concordance statistic; eGFR, estimated glomerular filtration rate; MELD, Model for End Stage Liver Disease; MELD(adj), MELD score with regression coefficients adjusted for our model; MELD(eGFR), MELD score with logeGFR substituted for logecreatinine; ROC, receiver-operating characteristic; UKELD, UK score for patients with End-stage Liver Disease ;UKELD(adj), UKELD score with regression coefficients adjusted for our model; UKELD(eGFR), UKELD score with logeGFR substituted for logecreatinine.

LogeGFR(MDRD6) substituted for logecreatinine did not change the concordance statistic for MELD as a predictor of 3-month waiting-list mortality (MELD(adj) vs MELD(eGFR), $p=0.825$). Furthermore, logeGFR(MDRD6) substituted for logecreatinine did not alter the concordance statistic for UKELD as a predictor of death by 3 months (UKELD(adj) vs UKELD(eGFR), $p=0.781$).

In view of the concern that the MELD and UKELD scoring systems are systemically bias and may discriminate against female and older patients the concordance statistics of individual patient groups were also determined (table 4.2). There was no statistically significant difference in the concordance statistics of the MELD score or UKELD score between genders (MELD, $p=0.718$; UKELD, $p=0.645$) and age groups (MELD, $p=0.099$; UKELD, $p=0.216$). LogeGFR(MDRD6) substituted for logecreatinine did not change the concordance statistic for MELD or UKELD as predictors of 3-month waiting-list mortality in females, males, older or younger patients (p values not shown).

Table 4.2: AUC for ROC curves for prediction of 3-month liver transplant waiting-list mortality in different patient groups.

Model	Females			Males			Older			Younger		
	c-statistic	95% CI		c-statistic	95% CI		c-statistic	95% CI		c-statistic	95% CI	
MELD	0.807	0.677-0.938		0.775	0.660-0.890		0.734	0.612-0.857		0.872	0.764-0.981	
MELD (adj)	0.847	0.740-0.955		0.820	0.723-0.917		0.810	0.703-0.916		0.882	0.797-0.967	
MELD (eGFR)	0.848	0.733-0.962		0.843	0.752-0.933		0.791	0.677-0.906		0.880	0.788-0.971	
UKELD	0.794	0.664-0.924		0.833	0.729-0.936		0.771	0.659-0.884		0.876	0.755-0.988	
UKELD (adj)	0.826	0.712-0.940		0.874	0.784-0.963		0.833	0.731-0.936		0.891	0.794-0.989	
UKELD (eGFR)	0.828	0.715-0.941		0.879	0.790-0.967		0.825	0.719-0.931		0.891	0.788-0.993	

Note: Older defined as age ≥60 years, Younger defined as age <60 years.

Abbreviations: CI, confidence interval; c-statistic, concordance statistic; eGFR, estimated glomerular filtration rate; MELD, standard Model for End stage Liver Disease; MELD(adj), MELD score with regression coefficients adjusted for our model; MELD(eGFR), MELD score with logeGFR substituted for logecreatinine; ROC, receiver-operating characteristic; UKELD, standard UK score for patients with End-stage Liver Disease; UKELD(adj), UKELD score with regression coefficients adjusted for our model; UKELD(eGFR), UKELD score with logeGFR substituted for logecreatinine.

Does substitution of CrCl for serum creatinine improve the prognostic accuracy of MELD and UKELD?

Measured creatinine clearance (CrCl) was available in 139 of the 256 patients (54.3%) listed for liver transplantation between May 2000 and June 2007. The CrCl patients were comparable to patients who did not have a recorded CrCl (table 4.3). In this cohort of 139, 31 patients (22.3%) died prior to transplantation. The median time from listing to death was 49 (IQR 19-88) days. The median waiting-time to transplantation was 85 (IQR 35-179) days. Fifty five patients (39.6%) were transplanted and 25 patients (18.0%) died within 3-months of listing.

Table 4.3: Comparison of listing variables in patients listed for liver transplantation between May 2000 and June 2007 who did and did not have CrCl available.

Variable	No CrCl (no:117)	CrCl (no: 139)	p value
Age (years)	54.0 (12.3)	55.3 (11.4)	0.374
Male gender	62(53.0)	85(61.2)	0.188
Noncholestatic disease	71(60.7)	83(59.7)	0.874
INR	1.4(1.2-1.6)	1.3(1.1-1.6)	0.553
Bilirubin (µmol/l)	76(42-139)	84(46-156)	0.526
Albumin (g/l)	28.9 (5.4)	29.0 (5.4)	0.876
Encephalopathy	36 (43.9)	35(42.7)	0.875
Ascites	60(60.6)	70(60.3)	0.969
Sodium (mmol/l)	135(132-138)	136(131-139)	0.591
Creatinine (µmol/l)	91(78-106)	91(79-106)	0.795
eGFR(MDRD6)	73(58-90)	76(60-87)	0.997
MELD	17(14-20)	16(14-21)	0.771
UKELD	57(54-61)	57(54-61)	0.936

Values expressed as mean (standard deviation), median (inter-quartile range) and number (percent) where appropriate.

Units for eGFR(MDRD6) = ml/min/1.73m².

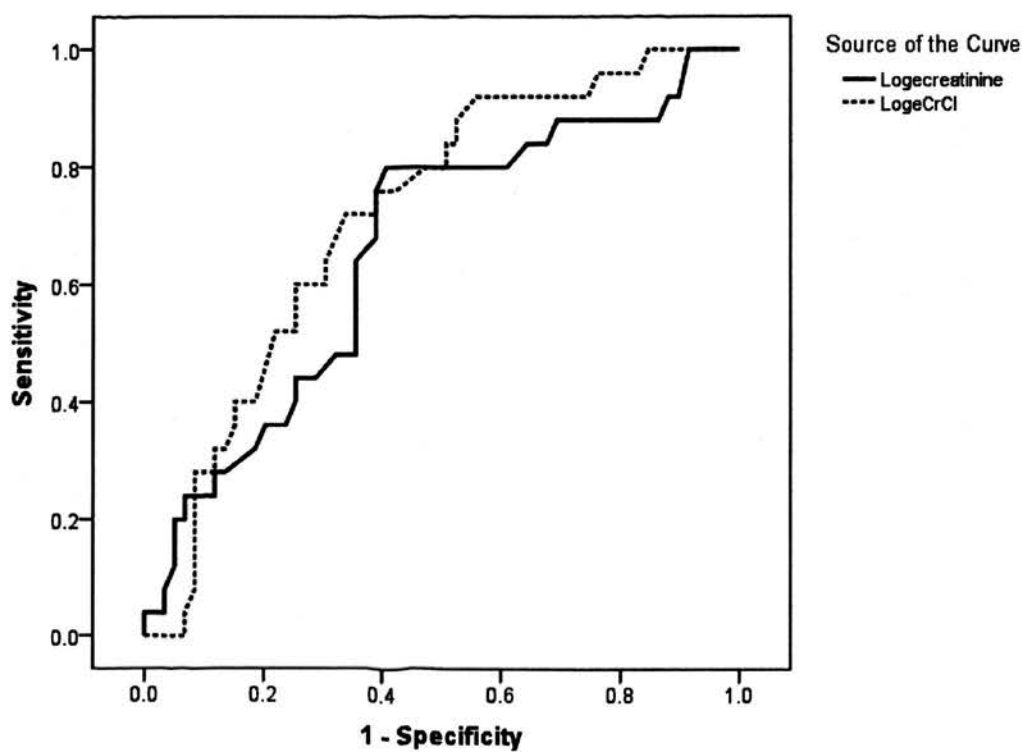
Abbreviations: CrCl, measured creatinine clearance; eGFR(MDRD6), estimated glomerular filtration rate derived from 6-variable MDRD equation; INR, international normalised ratio; MELD, Model for End stage Liver Disease; MDRD, Modification of Diet in Renal Disease; UKELD, UK score for patients with End-stage Liver Disease.

The median listing serum creatinine, serum sodium, eGFR(MDRD6) and CrCl was 91 (IQR 79-110) $\mu\text{mol/l}$, 136 (IQR 131-139) mmol/l , 75 (60-87) ml/min/1.73m^2 , and 72 (51-95) ml/min , respectively. CrCl demonstrated a greater correlation with eGFR(MDRD6) (0.615, $p<0.001$) than with serum creatinine (-0.452, $p<0.001$).

Logecreatinine (OR 7.77, 95% CI 1.33-45.51, $p=0.023$) and logeCrCl (OR 0.22, 95% CI 0.07-0.67, $p=0.008$) were associated with 3-month waiting-list mortality. ROC curves for logecreatinine and logeCrCl are shown in Figure 4.2. Logecreatinine and logeCrCl had similar concordance statistics for the prediction of death by 3 months (logecreatinine 0.660; 0.532-0.788: logeCrCl 0.718; 0.604-0.831, c-statistic and 95% confidence interval, $p=0.353$).

As before, ROC analysis was used to determine whether the substitution of logeCrCl for logecreatinine improved the accuracy of the existing prognostic models, MELD and UKELD (Table 4.4). LogeCrCl substituted for logecreatinine did not change the concordance statistic for MELD (MELD(adj) vs MELD(CrCl), $p=0.249$) or UKELD (UKELD(adj) vs UKELD(CrCl), $p=0.198$) as a predictor of 3-month waiting-list mortality.

Figure 4.2: ROC curves of log serum creatinine (logecreatinine) and log creatinine clearance (logeCrCl) for predicting 3-month liver transplant waiting list mortality.



Abbreviations: CrCl, measured creatinine clearance; ROC, receiver-operating characteristic.

Table 4.4: AUC for ROC curves for prediction of 3-month liver transplant waiting-list mortality.

Model	c-statistic	95% CI
MELD (adj)	0.809	0.708-0.910
MELD (CrCl)	0.845	0.765-0.926
UKELD (adj)	0.849	0.756-0.942
UKELD (CrCl)	0.881	0.808-0.954

Abbreviations: CI, confidence interval; CrCl, measured creatinine clearance; c-statistic, concordance statistic; MELD, Model for End stage Liver Disease; MELD(adj), MELD score with regression coefficients adjusted for our model; MELD(CrCl), MELD score with logeCrCl substituted for logecreatinine; ROC, receiver-operating characteristic; UKELD, UK score for patients with End-stage Liver Disease; UKELD(adj), UKELD score with regression coefficients adjusted for our model; UKELD(CrCl), UKELD score with logeCrCl substituted for logecreatinine.

4.5 Discussion

Our study has examined for the first time eGFR, calculated using the MDRD equations, in the prediction of mortality on the liver transplant waiting list. We have demonstrated that decreasing eGFR, as a continuous variable, was associated with an increased risk of death within 3 months of listing. This reiterates the well recognised spectrum of renal dysfunction that occurs in the setting of cirrhosis and reflects the underlying circulatory derangement of advanced disease. Of the three MDRD equations, the eGFR derived from the 6-variable equation was the better prognostic indicator. On univariate analysis, eGFR(MDRD6) was comparable, but not superior, to listing serum creatinine for prediction of 3-month waiting-list mortality. When substituted for serum creatinine eGFR(MDRD6) did not improve the prognostic accuracy of the existing MELD and UKELD models.

Although a negative study, the finding that eGFR(MDRD6) is not superior to serum creatinine in the prediction of waiting-list mortality is an important observation. Several studies have previously highlighted the prognostic inadequacies of serum creatinine in patients with end stage liver disease (414,415). Concerns have been raised that scoring systems for graft allocation that incorporate serum creatinine may disadvantage some individuals. In searching for alternative measures of renal function the next step is to use creatinine-based estimates of GFR that adjust for patient factors potentially conferring systemic bias. The MDRD eGFR is well validated in the non liver setting, is calculated from readily available variables including age, gender and race, and has been shown to be the most accurate eGFR in

cirrhotic patients (282,419,420,421). Our negative results support the need for further research to identify more precise non creatinine-based measures of renal function in these patients.

An explanation for the failure of eGFR(MDRD6) to improve the MELD and UKELD scoring systems is that the equation does not take into account disease-related factors such as nutritional status. Consequently, eGFR(MDRD6) is not an accurate measure of absolute renal function with one third of patients demonstrating an MDRD estimate outwith 30% of the measured GFR (419). The Cockcroft-Gault eGFR adjusts for body weight and, although a less precise estimator of glomular filtration rate in this population, it's ability to predict survival remains unknown (419,427). Notably, the difficulty obtaining an accurate dry weight in patients with significant ascites and peripheral oedema makes the Cockcroft-Gault eGFR a less attractive option (412,413).

Other possible weaknesses of eGFR for predicting mortality on the liver transplant waiting list are as follows. Similar to ascites and serum sodium concentration, eGFR may be influenced by diuretic use and could theoretically be subject to manipulation (299). Furthermore, a reduced eGFR may reflect intrinsic renal disease, which may not confer the same prognostic significance. All patients with evidence of renal impairment should have renal pathology excluded with urinalysis and renal imaging (105). Creatinine assays are not currently standardised and there is significant variability in serum creatinine levels using different methods (428). Therefore, the prognostic significance of eGFR may not be echoed in all centres.

The association of measured creatinine clearance with mortality in patients listed for liver transplantation was a further novel finding of this study. Decreasing measured creatinine clearance, as a continuous variable, was associated with an increased risk of death within 3 months of listing. Mirroring the findings of eGFR(MDRD6) measured creatinine clearance was a comparable, but not superior, prognostic indicator to serum creatinine. When substituted for serum creatinine measured creatinine clearance increased the accuracy of MELD and UKELD by 3.6% and 3.2%, respectively, although statistical significance was not achieved. The negative result may reflect a relatively small patient subgroup, but probably reflects the inaccuracy of measured creatinine clearance as a measure of absolute renal function (429).

Despite the large population assessed in this single-centre study, we recognise some potential limitations. Firstly, due to the retrospective nature we cannot ensure that all patients with intrinsic renal disease were excluded from the analysis. In our unit patients assessed for liver transplantation routinely undergo urine testing and renal ultrasonography, and those with significant renal impairment are considered for renal biopsy. As a result, most patients with intrinsic renal disease should have been identified. Secondly, biochemical values were based on a single measurement and may not have been a true representation of the steady state in all. However, during the 5-day liver transplant assessment our patients are relatively stable and less likely to be subject to diuretic-induced or sepsis-related acute renal impairment. Thirdly, the patients included in the study were listed over a 15 year period during which advances have been made in the management of CLD, such as the widespread use of

terlipressin and albumin for hepatorenal syndrome. Therefore, there may be a small time effect that could not be factored into the statistical analysis. Finally, the indications for transplantation in this cohort differ somewhat from the typical transplant centre with a greater proportion of patients listed for primary biliary cirrhosis and less for viral hepatitis. The MELD score has shown to have comparable 3-month mortality risk prediction in a diverse range of liver diseases, both cholestatic and non-cholestatic (410). Consequently, we do not believe that the somewhat atypical spread of aetiologies should have influenced our findings.

4.6 Conclusion

Clinically applicable, precise measures of renal function are not currently available in cirrhotic patients. Serum creatinine remains the most widely used parameter and despite its limitations has some clinical relevance. A change in serum creatinine may indicate haemodynamic decompensation or intrinsic renal disease, and serum creatinine is an important prognostic indicator (409,410). In this study we have demonstrated that listing eGFR(MDRD6) was comparable, but not superior, to listing serum creatinine for prediction of 3-month waiting-list mortality, and when substituted for serum creatinine eGFR(MDRD6) did not improve the prognostic accuracy of the existing MELD and UKELD models. Our findings support the need for further research to identify more precise non creatinine based measures of renal function.

CHAPTER 5

Implications of renal dysfunction for post liver transplant renal function: Incidence and risk factors for chronic kidney disease following liver transplantation for acute liver failure, and comparison with matched patients transplanted for chronic liver disease

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5.1 Introduction

Renal dysfunction is a common complication of ALF with two thirds of patients manifesting AKI, and almost half requiring RRT. Many have postulated that the pathogenesis is similar to the hepatorenal syndrome of cirrhosis (105,169). However, a growing body of evidence supports a systemic inflammatory response to ALF, and the SIRS is an independent predictor of AKI in ALF patients (175,215). It follows that the renal dysfunction of sepsis may be a more accurate parallel than the hepatorenal syndrome. Additional factors that may contribute to renal dysfunction in ALF but are less likely in stable cirrhotic patients include hypovolemia, nephrotoxic drugs particularly paracetamol, infection and disseminated intravascular coagulation (163,206,207).

Despite the contrasting peri-operative clinical condition of patients transplanted for ALF and CLD, post liver transplant renal outcomes have not been examined specifically in this group. Pre-transplant GFR, pre-transplant renal failure requiring RRT, and acute renal injury are consistent predictors of chronic renal dysfunction after elective liver transplantation (8,430). Given the greater baseline circulatory and neuro-humoral derangement of ALF it seems possible that the acute haemodynamic effects of the CNIs administered immediately following transplantation are exaggerated (263,264,279,280). On the other hand, the differing patho-physiological mechanisms could offer relative reno-protection and a reduced risk of CKD.

The clarification of the impact of liver transplantation for ALF on post transplant renal function has important implications for patient management. Chronic renal dysfunction is a major cause of patient morbidity and mortality and the minimisation of renal injury has emerged as a priority for transplant physicians (8,273,282,284). Simultaneous liver-kidney transplantation is not an option in patients transplanted for ALF because of the medical urgency, but the identification of prognostic variables could help to determine those who may benefit from tailored renal sparing immunosuppressive regimens (295,297).

5.2 Aims

The aims of this study were firstly to describe the incidence and risk factors for chronic renal dysfunction following liver transplantation for ALF and secondly to compare renal outcome with an age-sex-matched group of patients transplanted for CLD.

5.3 Methods

This was a retrospective single-centre study of consecutive patients who underwent super-urgent liver transplantation for ALF (UK Transplant Super Urgent Scheme Category 1-7) between December 1992 and July 2007 (431). Eight patients had inadequate documentation available and were excluded from the analysis. A further 1 patient was lost to follow-up. Therefore, the study cohort comprised 101 patients.

ALF was defined as severe liver injury with hepatic encephalopathy in which the onset of encephalopathy was within 8 weeks for the first symptoms of illness, and in the absence of pre-existing liver disease (159).

Data was collected on the following pre-operative variables at the time of listing: age, gender, race, liver disease aetiology, additional co-morbidity, smoking status, INR, serum bilirubin, albumin, serum creatinine, serum sodium (hyponatraemia; sodium <135 mmol/l), and presence of ascites (on ultrasound). SIRS was defined as ≥ 2 of temperature $<36^{\circ}\text{C}$ or $>38^{\circ}\text{C}$, heart rate >90 beats per minute, WCC $<4 \times 10^9/\text{l}$ or $>12 \times 10^9/\text{l}$, and $\text{PaCO}_2 < 4.3 \text{ kPa}$ at the time of admission (212). Documented peri-operative variables were peak pre-operative serum creatinine, pre-operative RRT, post-operative RRT, inotropes (noradrenaline/adrenaline), bacterial sepsis and fungal sepsis. Immunosuppression was noted and CNI trough levels at 1-week, 1-month and 12-months (a comparable 12-month value for the linear regression analysis was obtained for all patients regardless of CNI by expressing the trough as relative to the median value). Renal function was recorded at 1-month, 6-months, 12-months, and

2-, 3-, 4- and 5-years following transplantation. Patients still receiving RRT at 1-month were given an arbitrary serum creatinine of 350 $\mu\text{mol/l}$ and an eGFR of 15 ml/min/1.73m^2 .

A patient was considered to have significant renal dysfunction pre-operatively if they fulfilled the RIFLE criteria for AKI: peak serum creatinine ≥ 2 times the baseline level (390). The baseline serum creatinine was unavailable for most patients and was estimated as previously described (390). Following transplantation the main measure of renal function was eGFR, determined using the MDRD Study 4-variable equation (421).

$$\text{eGFR} = 186 \times \text{creatinine}(\text{mg/dl})^{-1.154} \times \text{age}(\text{years})^{-0.203} \times 1.210(\text{if black}) \times 0.742(\text{if female})$$

CKD was defined as eGFR $< 60 \text{ ml/min/1.73m}^2$ on at least 2 occasions from 6 months post transplant onwards: stage 3, stage 4 and stage 5 CKD were defined as eGFR 30-59 ml/min/1.73m^2 , 15-29 ml/min/1.73m^2 , and $< 15 \text{ ml/min/1.73m}^2$ or on dialysis, respectively (282).

To examine whether the renal dysfunction of ALF has a different renal prognosis after transplantation to the renal dysfunction of CLD a control group of patients transplanted for CLD was identified. These patients were age-matched (to within 5 years) and sex-matched to the original cohort. The relatively young age of the patients transplanted for ALF meant that only 71 patients could be appropriately

matched. The causes of CLD were primary biliary cirrhosis (18 patients, 25.4%), alcohol (10 patients, 14.1%), chronic active hepatitis (9 patients, 12.7%), sclerosing cholangitis (9 patients, 12.7%), cryptogenic cirrhosis (9 patients, 12.7%), hepatitis C (5 patients, 7.0%) and other (11 patients, 15.5%). Three patients (4.2%) were transplanted for hepatocellular carcinoma. None of the control patients had intrinsic renal disease prior to transplantation and no patient underwent combined liver-kidney transplantation.

Immunosuppression was similar for patients transplanted for ALF and for CLD, and consisted of a CNI, azathioprine and prednisolone in most cases. Midway through the specified time period the unit policy for CNI changed from cyclosporine to tacrolimus. Prednisolone was usually discontinued by 3 to 6 months post transplant unless otherwise indicated. Deviation from the protocol occurred only in the setting of adverse event or graft rejection. Acute rejection was usually managed with 1g of methyl-prednisolone intravenously for 3 days followed by re-introduction of oral steroids with or without increased dose of, or switch to, alternative CNI. Chronic rejection was managed with the latter and in a small number of patients azathioprine was changed to mycophenolate. IL-2 receptor antagonist induction therapy was not administered to any of the patients.

Statistical analyses

Cumulative incidence of CKD was estimated using the Kaplan-Meier method. Survival was estimated using Kaplan-Meier plots with log-rank test for differences, and age-adjusted survival was determined using Cox proportional hazards analyses. Normally distributed continuous variables and non-parametric continuous variables were compared using the Student's t-test and Mann-Whitney test, respectively. Chi-squared analysis or Fisher's exact test were used for comparison of categorical data. A multivariate linear regression analysis was performed to explore the relationship between peri-operative renal dysfunction and long term renal function following transplantation. Clinically relevant factors were included simultaneously with 12-month eGFR as the dependent variable. Cox proportional hazards analysis was then used to identify variables predictive of CKD by 5-years post transplant. Three multivariate models were constructed with all clinically relevant factors entered simultaneously. Variables entered into Model 1 were age, gender, pre-transplant diagnosed hypertension, category of ALF (paracetamol-induced vs non-paracetamol-induced), SIRS, CNI at time of hospital discharge and pre-transplant AKI. In Models 2 and 3 AKI was replaced by the other measures of peri-operative renal dysfunction, peak pre-operative change in serum creatinine and immediate post transplant RRT, respectively. All 3 measures of peri-operative renal dysfunction were not included in the same model because of collinearity. None of the multivariate models were adjusted for the presence of pre-transplant diabetes mellitus secondary to small patient numbers. $P < 0.05$ was considered statistically significant unless otherwise stated. Data was analysed using the SPSS 15 package.

All values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

5.4 Results

Patient characteristics

The causes of ALF were paracetamol (46 patients, 45.5%), seronegative hepatitis (27 patients, 26.7%), idiosyncratic drug reaction (11 patients, 10.9%), autoimmune hepatitis (7 patients, 6.9%), hepatitis B (4 patients, 4.0%), Budd-Chiari (3 patients, 3.0%), Wilsons disease (2 patients, 2.0%) and hepatitis A (1 patient, 1.0%).

The median jaundice to encephalopathy time for patients with non-paracetamol-induced ALF was 14 (IQR 11-31) days. In patients with paracetamol-induced ALF the median time from overdose to listing for liver transplantation was 69 (IQR 54-72) hours. Patient characteristics at the time of listing are outlined in Table 5.1. The median time from listing to transplantation was 1 (IQR 1-2) day. The estimated 1-month, 12-month and 5-year post transplant patient survival was 83%, 75% and 68%, respectively.

Table 5.1: Clinical characteristics of ALF patients at time of listing for liver transplantation.

Clinical characteristics	(no:101)
Baseline demographics	
Age (years)	36.3(14.1)
Male:Female	1:1.5
Caucasian	99(98)
Comorbidity	
Diagnosed hypertension	6(5.9)
Type I diabetes mellitus	2(2.0)
Type II diabetes mellitus	1(1.0)
Dyslipidaemia	1(1.0)
Cause of ALF	
Paracetamol	46 (45.5)
Seronegative hepatitis	27 (26.7)
Idiosyncratic drug reaction	11 (10.9)
Autoimmune hepatitis	7 (6.9)
Hepatitis B	4 (4.0)
Budd-Chiari	3 (3.0)
Wilson's disease	2 (2.0)
Hepatitis A	2 (1.0)
Clinical characteristics at listing	
Bilirubin ($\mu\text{mol/l}$)	193(80-463)
INR	8.3(3.2-11.7)
Albumin (g/l)	30.8(10.2)
Creatinine ($\mu\text{mol/l}$)	160(94-298)
Sodium (mmol/l)	135(5)
MELD score	43(35-52)
Ascites	24(23.8)
SIRS	59(70.2)
Grade III/IV encephalopathy	57(59.4)
Inotropes	36(37.9)

Values expressed as mean (standard deviation), median (inter-quartile range) and number (percent) where appropriate.

Abbreviations: ALF, acute liver failure; INR, international normalised ratio; MELD, Model for End-stage Liver Disease; SIRS, systemic inflammatory response syndrome.

Peri-operative renal function

Actual baseline renal function was available in 21 patients: the median baseline serum creatinine was 75 (IQR 60-93) $\mu\text{mol/l}$ and the mean baseline eGFR was 106 (SD 45) ml/min/1.73m^2 .

During the immediate pre-operative period the median peak serum creatinine of the entire cohort was 203 (IQR 102-362) $\mu\text{mol/l}$. Fifty-four patients (53.5%) fulfilled the criteria for AKI, of whom 72.2% underwent RRT. A further 5 patients were commenced on haemofiltration in the absence of a creatinine rise. Following transplantation 64.9% (n=63) received RRT. By 1-month post transplant the median serum creatinine was 97 (IQR 83-136) $\mu\text{mol/l}$ and the mean eGFR was 67 (SD 40) ml/min/1.73m^2 . Four of the surviving patients (4.8%) were still on RRT at this time point.

When patients with and without paracetamol-induced ALF were compared the former were more likely to demonstrate peri-operative renal dysfunction. Paracetamol-induced ALF patients had a greater median peak pre-operative serum creatinine (paracetamol-induced-ALF, 332 (217-415) $\mu\text{mol/l}$, n=47; non-paracetamol-induced-ALF, 108 (86-187) $\mu\text{mol/l}$, median (IQR), n=54; $p<0.001$), a greater frequency of AKI (paracetamol-induced-ALF, 83%; non-paracetamol-induced-ALF, 28%; $p<0.001$), and a greater frequency of pre- (paracetamol-induced-ALF, 74%; non-paracetamol-induced-ALF, 15%; $p<0.001$) and post-operative RRT (paracetamol-induced-ALF, 95%; non-paracetamol-induced-ALF, 38%; $p<0.001$). At 1-month post transplant mean eGFR was similar for the two groups (paracetamol-

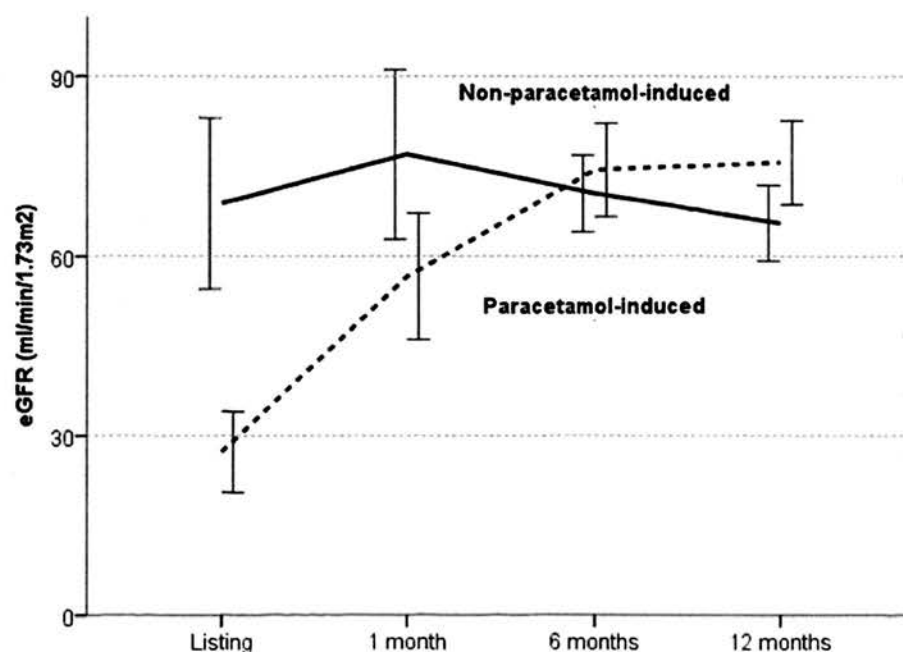
induced-ALF, 57 (30) ml/min/1.73m², n=36; non-paracetamol-induced-ALF, 74 (44) ml/min/1.73m²; mean (SD), n=48; p=0.053).

Post-operative renal function

In most patients renal function demonstrated maximal recovery by 6- to 12-months following transplantation. The mean 12-month eGFR was 70 (SD 21) ml/min/1.73m², and 21.1% (n=16) of patients had stage 3-5 CKD by this time point. In those patients with follow up to 5 years after transplantation the mean eGFR remained stable at 70 (SD 20) ml/min/1.73m², and the prevalence of stage 3-5 CKD was 29.5% (n=13). Twelve month eGFR demonstrated a close correlation with 5-year eGFR (r=0.809, p<0.001). The cumulative incidence of stage 3-5, and stage 4-5 CKD by 5-years was 41.5% and 2.6%, respectively.

Figure 5.1 demonstrates the mean listing and 1-month, 6-months and 12-months after transplantation eGFR of patients surviving to 12-months, subdivided based on paracetamol as the cause for ALF compared with other aetiologies. Beyond the peri-operative period, there was no difference in the mean eGFR of the paracetamol-induced ALF and non-paracetamol-induced ALF groups (p value <0.013 considered significant). The cumulative incidence of stage 3-5 CKD by 1- and 5-years post transplant was 8.6% and 27.4% for paracetamol-induced ALF patients respectively, and 29.3% and 50.9% respectively for non-paracetamol-induced ALF patients (p=0.021).

Figure 5.1: Mean eGFR and 95% confidence intervals at the time of listing for liver transplantation and at 1, 6 and 12 months following transplantation in all patients surviving to 12 months subdivided into paracetamol-induced and non-paracetamol-induced ALF groups. P value <0.013 considered significant.



eGFR (ml/min/1.73m²) compared at time points

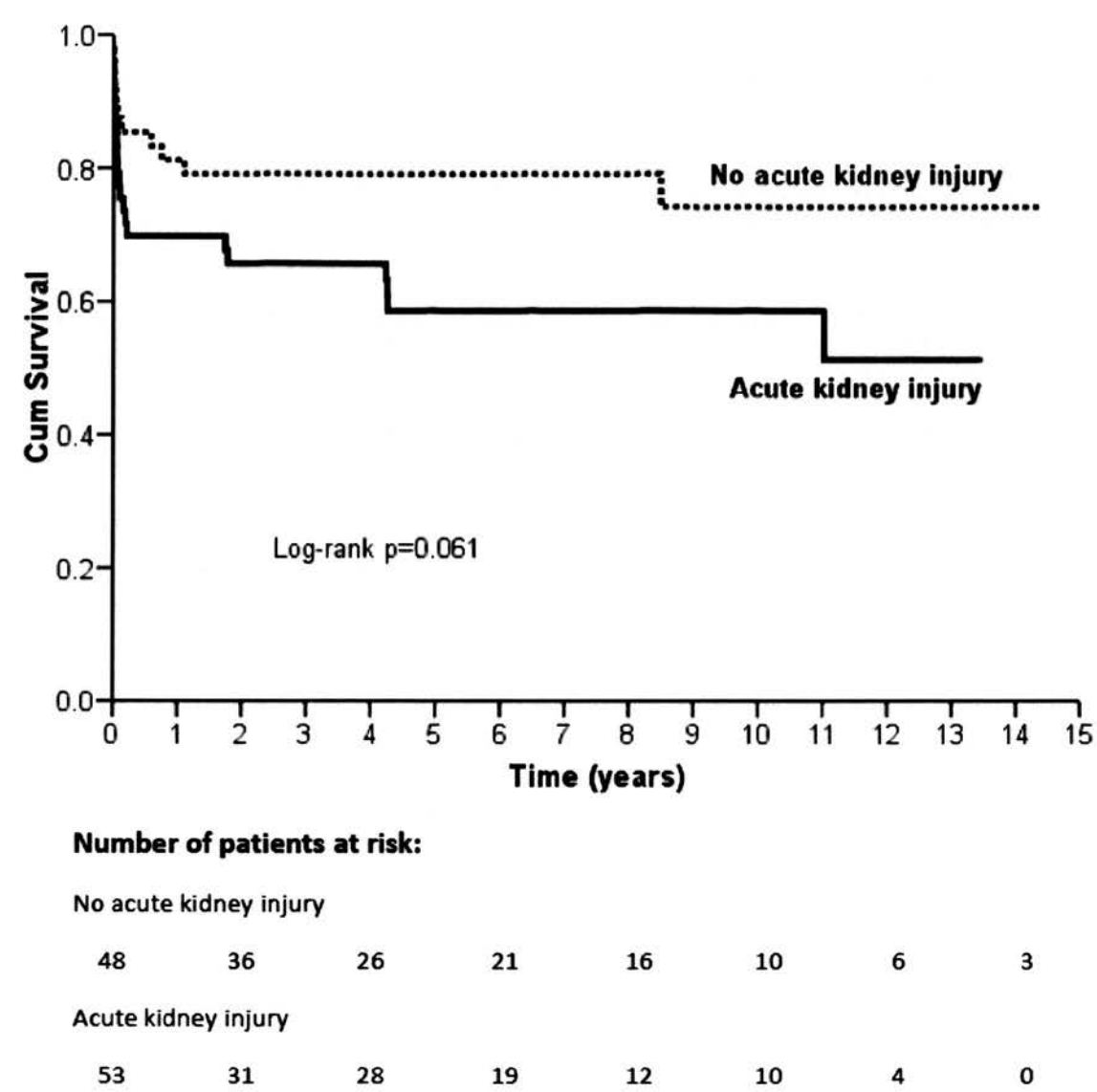
Non-paracetamol-induced (n:41)	68.8	77.0	70.5	65.5
Paracetamol-induced (n:35)	27.2	56.6	74.4	75.7
P value	<0.001	0.026	0.429	0.032

Abbreviations: ALF, acute liver failure; eGFR, estimated glomerular filtration rate.

Relationship between peri-operative renal dysfunction and post transplant mortality

ALF patients who fulfilled the criteria for AKI prior to transplantation had greater mortality post transplant (log-rank $p=0.061$; age-adjusted HR 2.09; 95% CI 1.01-4.34, $p=0.048$) (Figure 5.2). Similarly, patients who required post operative RRT demonstrated an increased risk of death (age-adjusted HR 6.22; 95% CI 2.01-19.26, $p=0.002$).

Figure 5.2: Kaplan-Meier plot of the probability of survival following liver transplantation for ALF subdivided based on the presence or absence of pre-operative AKI.



Abbreviations: AKI, acute kidney injury; ALF, acute liver failure.

Relationship between peri-operative renal dysfunction and post transplant renal function

To explore the relationship between peri-operative renal dysfunction and long term renal function following transplantation for ALF a multiple linear regression analysis was performed, therefore, allowing adjustment for other relevant clinical factors such as age, gender and immunosuppressive therapy. Given the close correlation between 12-month eGFR and 5-year eGFR, 12-month eGFR was used as the dependent variable (Table 5.2). The analysis revealed no significant association between pre-transplant AKI and 12-month post transplant eGFR ($p=0.098$). Instead, increasing age ($p=0.012$), female gender ($p=0.005$), pre-operative SIRS ($p=0.041$) and cyclosporine as primary immunosuppression ($p=0.021$) were associated with worse renal function. Patients with paracetamol-induced ALF had a higher 12-month eGFR compared with patients with non-paracetamol-induced ALF ($p=0.027$).

Table 5.2: Multivariate linear regression analysis of variables associated with eGFR 12-months following liver transplantation for ALF.

	B	(95% CI)	β	p value
Age (years)	-0.516	-0.916, -0.116	-0.318	0.012
Female gender	-14.500	-24.358, -4.643	-0.332	0.005
Past medical history: hypertension	-20.354	-59.134, 18.426	-0.123	0.297
Paracetamol-induced ALF	16.176	1.889, 30.463	0.371	0.027
Pre-operative SIRS	-12.780	-24.991, -0.570	-0.270	0.041
Pre-operative acute kidney injury	-10.204	-22.369, 1.960	-0.235	0.098
CNI: cyclosporine	-12.465	-22.947, -1.983	-0.263	0.021
12-month CNI trough	0.583	-7.662, 8.832	0.016	0.887

Reference group (relative risk 1.00): male gender, past medical history: no hypertension, non-paracetamol-induced ALF, no pre-operative SIRS, no pre-operative acute kidney injury CNI: tacrolimus (at time of 12-month eGFR).

Abbreviations: ALF, acute liver failure; B, unstandardised regression coefficient; β , standardised regression coefficient; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; SIRS, systemic inflammatory response syndrome.

Predictors of chronic kidney disease following transplantation

Recognising the significant morbidity and mortality of CKD, as well as concerns regarding the influence of early deaths after transplantation on the 12-month data, cox regression was then performed to identify peri-operative variables predictive of post transplant CKD. Variables associated with the development of CKD following transplantation on univariate analysis are outlined in table 5.3. A subsequent multivariate regression analysis including all clinically relevant variables simultaneously (Model 1 table 5.3) identified older age (overall $p=0.019$), female gender ($p=0.049$), pre-transplant diagnosed hypertension ($p=0.031$) and cyclosporine immunosuppressive therapy ($p=0.027$) to be predictors of CKD after transplantation. Patients transplanted for paracetamol-induced ALF were at lower risk of CKD than patients transplanted for non-paracetamol-induced ALF ($p=0.039$).

Pre-transplant AKI both on univariate analysis ($p=0.796$), and after adjusting for confounding factors ($p=0.288$), was not predictive of post transplant CKD. Similarly, no relationship was demonstrated between peak pre-operative change in serum creatinine (univariate analysis, $p=0.838$; multivariate analysis, $p=0.457$, Model 2 table 5.3), or RRT during the immediate post transplant period (univariate analysis, $p=0.420$; multivariate analysis, $p=0.134$, Model 3 table 5.3), and chronic renal dysfunction.

Table 5.3: Univariate and multivariate cox regression analysis of variables as predictors of CKD (eGFR <60 ml/min/1.73m²) by 5-years following liver transplantation for ALF.

	Univariate		Multivariate Model 1		Multivariate Model 2		Multivariate Model 3	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age	1.00		1.00		1.00		1.00	
<30 years								
30-44 years	3.59 (1.10-11.67)	0.034	4.14 (1.18-14.50)	0.026	4.18 (1.19-14.65)	0.026	4.32 (1.27-14.62)	0.019
≥45 years	11.35 (3.63-35.51)	<0.001	5.94 (1.62-21.74)	0.007	6.11 (1.67-22.36)	0.006	8.15 (2.08-31.96)	0.003
Female gender	2.23 (0.95-5.25)	0.067	2.94 (1.00-8.64)	0.049	2.86 (0.96-8.58)	0.060	2.92 (1.02-8.34)	0.045
Hypertension	2.39 (0.57-10.11)	0.236	14.16 (1.28-156.2)	0.031	12.17 (1.12-131.89)	0.040	12.95 (1.20-140.22)	0.035
ALF	1.00		1.00		1.00		1.00	
Non POD								
POD	0.42 (0.18-0.95)	0.037	0.23 (0.06-0.93)	0.039	0.26 (0.07-1.01)	0.052	0.22 (0.06-0.81)	0.022
Pre-operative:								
SIRS	1.41 (0.56-3.52)	0.467	2.11 (0.63-7.12)	0.229	2.09 (0.63-7.01)	0.231	1.93 (0.57-6.52)	0.288
Acute kidney injury	0.91 (0.43-1.91)	0.796	1.70 (0.64-4.51)	0.288				
Peak Δ creatinine	0.97 (0.75-1.26)	0.838			1.14 (0.81-1.61)	0.457		
Post-operative:								
CNI on discharge	1.00		1.00		1.00		1.00	
Tacrolimus								
Cyclosporine	2.00 (0.95-4.19)	0.067	2.67 (1.12-6.37)	0.027	2.67 (1.12-6.36)	0.026	2.20 (0.88-5.48)	0.091
RRT	0.74 (0.35-1.55)	0.420					2.39 (0.77-7.44)	0.134
1 month eGFR	1.00							
(ml/min/1.73m ²)								
≥90								
60-89	11.63 (1.50-90.24)	0.019						
30-59	12.02 (1.52-95.17)	0.018						
<30	18.95 (2.33-154.4)	0.006						

Reference group (relative risk 1.00): male gender, past medical history: no hypertension, no SIRS, no acute kidney injury, no RRT.
Abbreviations: ALF, acute liver failure; CKD, chronic kidney disease; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; peak Δ creatinine, peak change in serum creatinine (peak serum creatinine / baseline serum creatinine); RRT, renal replacement therapy during immediate post-operative period; SIRS, systemic inflammatory response syndrome.

Comparison of post transplant renal function in patients transplanted for acute liver failure and age-sex matched patients transplanted for chronic liver disease

To determine whether ALF per se is associated with renal function following transplantation age-sex-matched patients transplanted for CLD were introduced into the statistical analysis. Pre- and peri-operative characteristics of patients with ALF and with CLD are compared in table 5.4. The median waiting-list time was 1 (IQR 1-2) day for ALF patients and 52 (IQR 20-136) days for CLD patients ($p<0.001$). Median listing serum creatinine was higher ($p<0.001$), mean listing eGFR lower ($p<0.001$) and the frequency of ascites less ($p<0.001$) in the ALF group. Furthermore, the ALF patients were more likely to receive pre- ($p<0.001$) and post-operative RRT ($p<0.001$). The estimated 1-month, 12-month and 5-year survival was 78%, 69%, and 62%, respectively, for ALF patients and 96%, 90% and 79% for patients with CLD (Figure 5.3, log-rank $p=0.029$).

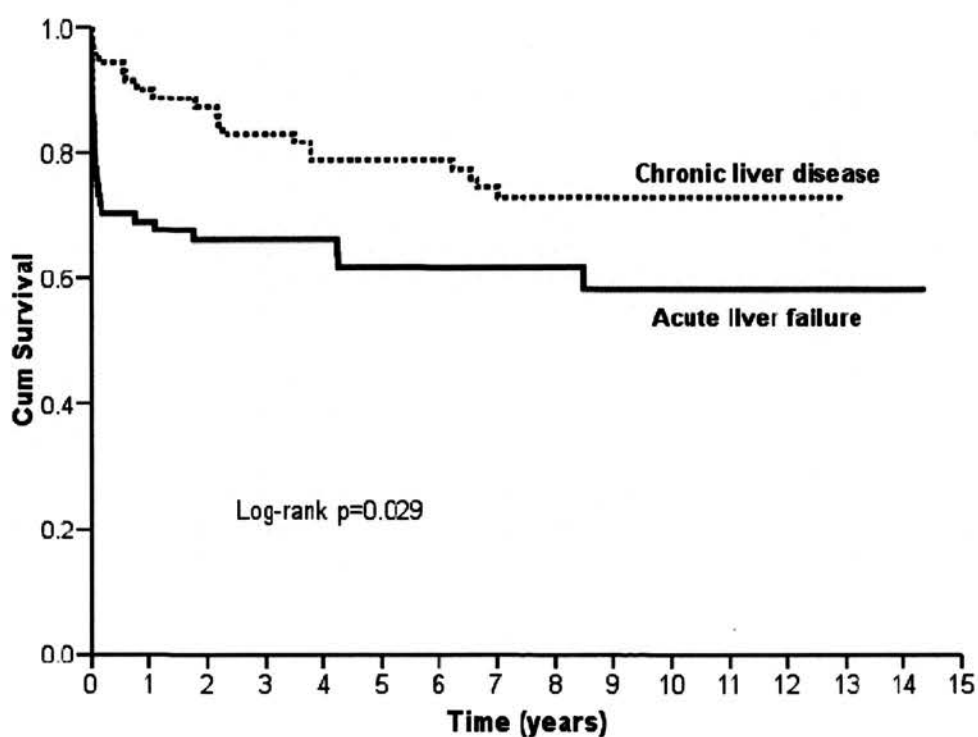
Table 5.4: Pre- and peri-operative clinical characteristics of patients transplanted for ALF and age-sex-matched patients transplanted for CLD.

	ALF (no:71)	CLD (no:71)	p value
Age (years)	42.2(12.4)	42.5(12.1)	0.918
Male:Female	1:1.3	1:1.3	1.000
Time on waiting list (days)	1(1-2)	52(20-136)	<0.001
Comorbidity			
Diagnosed hypertension	6(8.5)	2(2.8)	0.137
Type I diabetes mellitus	0(0)	(0)	1.000
Type II diabetes mellitus	1(1.4)	5(7.0)	0.104
Dyslipidaemia	1(1.4)	4(5.6)	0.183
Hepatitis C	1(1.4)	6(8.5)	0.058
Active smoker	37(56.9)	20(29.4)	0.001
At listing			
INR	5.8(2.8-11.1)	1.2(1.1-1.5)	<0.001
Bilirubin (µmol/l)	227(81-486)	67(35-172)	<0.001
Albumin (g/l)	30.8(11.4)	30.0(5.6)	0.582
MELD score	41(33-52)	16(12-19)	<0.001
Creatinine (µmol/l)	148(94-298)	81(71-97)	<0.001
eGFR (ml/min/1.73m ²)	49(42)	83(30)	<0.001
Hyponatraemia	27(38.0)	19(26.8)	0.151
Ascites	18(25.4)	41(57.7)	<0.001
Peri-operative			
Peak creatinine (µmol/l)	200(99-384)		
Pre RRT	30(42.3)	0(0)	<0.001
Post RRT	41(61.2)	12(16.9)	<0.001
Fungal sepsis	5(7.5)	3(4.2)	0.327
Bacterial sepsis	35(52.2)	25(35.2)	0.044
Super-urgent retransplant	4(5.6)	4(5.6)	0.641
Early acute cellular rejection	17(25.4)	28(39.4)	0.078
CNI on discharge: cyclosporin	16(28.1)	27(39.1)	0.192

Values expressed as mean (standard deviation), median (inter-quartile range) and number (percent) where appropriate.

Abbreviations: ALF, acute liver failure; CLD, chronic liver disease; CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; INR, international normalised ratio; MELD, model for end-stage liver disease; RRT, renal replacement therapy.

Figure 5.3: Kaplan-Meier plot of the probability of survival following liver transplantation for ALF and CLD.



Number of patients at risk:

Chronic liver disease

71	62	56	56	33	13	1	0
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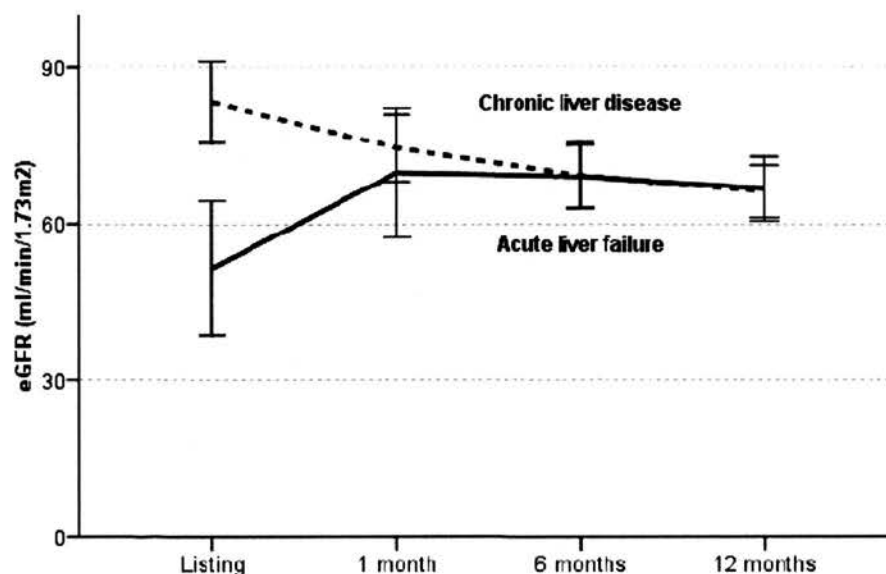
Acute liver failure

71	43	32	23	26	11	4	1
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Abbreviations: ALF, acute liver failure; CKD, chronic liver disease.

By 1 month following transplantation the median serum creatinine (ALF, 92 (83-127) $\mu\text{mol/l}$; CLD, 88 (79-101) $\mu\text{mol/l}$, median (IQR); $p=0.103$) and the mean eGFR (ALF, 69 (42) ml/min/1.73m^2 ; CLD, 74 (27) ml/min/1.73m^2 , mean (SD); $p=0.451$) were similar in ALF and CLD patients. Figure 5.4 illustrates the mean pre- and post-transplantation eGFR in patients with ALF and CLD surviving to 12 months. The accompanying table (table 5.5) documents relevant pre- and post-transplant clinical variables of these surviving patients. Despite significantly lower listing eGFR in the ALF group renal function was similar at all time points in the post-operative period. The cumulative incidence of stage 3-5 (ALF, 48.7%; CLD, 49.6%; $p=0.930$) and stage 4-5 CKD (ALF, 4.1%; CLD, 8.7%; $p=0.615$) by 5 years was also no different between ALF and CLD groups.

Figure 5.4: Mean eGFR and 95% confidence intervals at the time of listing for liver transplantation and at 1, 6 and 12 months following transplantation in all patients surviving to 12 months subdivided into ALF and CLD groups. P value <0.013 considered significant. The median time from listing to transplantation for ALF patients was 1 (IQR 1-2) day and for CLD patients was 52 (20-136) days.



eGFR (ml/min/1.73m²) compared at time points

Chronic liver disease (n:62)	83.3	74.5	69.2	66.2
Acute liver failure (n:49)	51.1	69.8	69.0	66.7
p value	<0.001	0.468	0.965	0.897

Abbreviations: ALF, acute liver failure; CLD, chronic liver disease; eGFR, estimated glomerular filtration rate.

Table 5.5: Relevant pre- and post-transplant clinical characteristics of all patients transplanted for ALF and patients transplanted for CLD surviving to 12 months after transplant.

Characteristic	ALF patients (no:49)	CLD patients (no:62)	p value
Age at listing (years)	41.8(12.4)	42.5(12.4)	0.772
Male:Female	1:1	1:1.3	0.569
Comorbidity at 12-month post transplant			
Diagnosed hypertension	14(28.6)	17(27.4)	0.893
Type II diabetes mellitus	7(14.3)	7(11.3)	0.637
Insulin-dependent diabetes mellitus	1(2.0)	6(9.7)	0.103
CNI on discharge: cyclosporine	16(32.7)	25(40.3)	0.406
1 week CNI trough:			
Cyclosporine	181(57)	145(52)	0.157*
Tacrolimus	8.5(3.3)	10.1(3.1)	0.195*
1 month CNI trough:			
Cyclosporine	151(39)	162(48)	0.492*
Tacrolimus	10.8(4.6)	8.1(3.2)	0.006*
12 month CNI trough:			
Cyclosporine	133(40)	160(40)	0.073*
Tacrolimus	8.0(3.3)	7.9(2.8)	0.974*

Values expressed as mean (standard deviation) and number (percent) where appropriate.

*P value <0.017 considered significant.

Abbreviations: ALF, acute liver failure; CLD, chronic liver disease; CNI, calcineurin inhibitor.

5.5 Discussion

In this large single-centre study we have described for the first time the risk factors for chronic renal dysfunction following emergency liver transplantation for ALF. Importantly, we have shown that peri-operative kidney injury does not appear to have negative consequences for long term renal function in this population. Contrary to observations in CLD patients pre-transplant AKI and RRT were not associated with CKD. Only failure to recover renal function, as evidenced by eGFR at 1-month post transplant, was a predictive factor. Despite marked differences in the peri-operative clinical condition of patients transplanted for ALF and CLD long term renal outcome following transplantation was the same.

The rate of CKD after transplantation for ALF was similar to that reported by Aberg et al, the single other publication of renal function in this setting (432). Although half of our patients fulfilled the criteria for AKI pre-transplant, and more than 60% required RRT during the immediate post-operative period, only 21% had an eGFR less than 60ml/min/1.73m^2 12 months thereafter. This dramatic renal recovery echoes clinical observations in spontaneous survivors of ALF. By 5-years post transplant the cumulative incidence of CKD was 42%.

The identical post transplant renal function of ALF and CLD patients was unexpected when considering our current understanding of the underlying mechanisms (4,263,264,279,280). Based on the traditional hypothesis of hepatorenal syndrome one might predict that severe peri-operative renal vasoconstriction would

exacerbate calcineurin-inhibitor mediated kidney dysfunction (4,263,264,279,280). Cyclosporine and tacrolimus cause an initially haemodynamic dose-dependent renal impairment that is feasibly exaggerated in patients with greater baseline circulatory and neuro-humoral derangement (263,264,279,280). Our results support an alternative patho-physiological process underlying the renal injury that occurs in ALF.

In Chapter 3 we demonstrated that SIRS predicts the development of AKI in patients with non-paracetamol-induced ALF, a relationship that appears to be independent of the severity of liver injury. Consequently, we have postulated that the renal dysfunction of sepsis may be a more accurate parallel than the hepatorenal syndrome of cirrhosis. In fulminant hepatic failure the systemic inflammatory response may be the key mediator of renal impairment. Patients with subfulminant ALF are more likely to have clinically significant portal hypertension, and may develop ascites (172). Therefore, this group may share some of the haemodynamic and neuro-humoral features of hepatorenal syndrome. In sepsis, kidney injury may occur in the setting of preserved or even increased renal perfusion, which is in contrast to the intense renal vasoconstriction of hepatorenal syndrome (4,183). We propose that relative renal hyperaemia may help to minimise the renal haemodynamic response to CNIs and explain the comparable long term post transplant renal function demonstrated by ALF patients (263).

Alternatively, the failure of peri-operative renal dysfunction to impact on long term post transplant renal outcomes may reflect the duration of renal impairment. In

patients transplanted for CLD renal dysfunction duration appears to be a key determinant of chronic renal impairment. Campbell et al demonstrated that renal dysfunction duration of greater than 3.6 weeks pre-transplant was an appropriate cut-off to identify patients at risk of renal insufficiency 12-months thereafter (433). In our cohort the renal injury, although more severe, was on the contrary short-lived.

In the non-transplant population AKI is a risk factor for chronic renal dysfunction. For example, in patients who undergo major vascular surgery the occurrence of peri-operative AKI is associated with an increased risk of CKD (268). Furthermore, patients requiring dialysis for AKI who are dialysis-independent at the time of hospital discharge are 3 times more likely to develop end-stage renal failure (269). Animal studies have confirmed that AKI can cause permanent structural kidney damage with progressive tubulo-interstitial fibrosis and long term implications for renal function (270). Our failure to show a relationship between peri-operative renal dysfunction and post transplant CKD is not in accordance with these observations. AKI is an independent predictor of mortality in patients with ALF, and following transplantation for ALF. Yet, our findings suggest that beyond hospital discharge acute renal impairment, if short-lived, does not impact particularly on chronic renal function.

Paracetamol as the cause of ALF was associated with a higher absolute eGFR at 12-months following transplantation and a reduced risk of CKD. Paracetamol is an independent predictor of AKI in patients with ALF and there are case reports of renal failure following paracetamol overdose in the absence of significant hepatic injury

(209,211). Animal models support a direct nephrotoxic effect although the mechanism remains unclear (209). It has been hypothesised that a locally produced metabolite induces proximal tubular cell necrosis while functional renal effects may also contribute (209,210,400). Our findings support the reversibility of paracetamol-induced nephrotoxicity (434,435).

The study has some potential limitations that should be mentioned. Firstly, baseline renal function was only available in a small number of ALF patients and it is possible that a proportion could have had undiagnosed intrinsic renal disease. The patients studied were of a relatively young age and it is assumed that pre-morbid renal function was normal. Secondly, nephrotoxic medications could have influenced the severity of peri-operative renal dysfunction. Our unit avoids nephrotoxic drugs, yet, this does not preclude exposure prior to transfer. Thirdly, although our study consists of one of the largest single centre cohorts of patients transplanted for ALF it remains possible that the relatively small numbers may have influenced our results.

With regards the CLD group, only 70% of the ALF patients could be matched because of the young age range. Furthermore, the pre-transplant eGFR was only available at the time of listing and not immediately prior to transplantation. Pre-transplant kidney function may, therefore, have been over represented in the CLD patients if there was a significant deterioration on the list. Nevertheless, no CLD patient required pre-operative RRT or reassessment for combined liver-kidney transplantation and, given the relatively short median waiting-list time of 52 days, it seems unlikely that this data would have influenced the results. The lack of pre-

transplant renal impairment in the control arm may also raise some concerns about its generalisability for a standard population of liver transplant recipients. This largely reflects the younger age of the patients. However, those with intrinsic renal disease or who received a simultaneous liver-kidney transplant were also deliberately excluded; we wished to examine whether the physiological differences between ALF and CLD would influence renal outcomes. Of course, it is well recognised that eGFR is not an accurate measure of renal function in patients listed for elective liver transplantation, tending to overestimate when the true GFR is reduced (419). Sixty percent of the CLD patients had ascites and one third had hyponatraemia, indicating a high prevalence of portal hypertensive-related renal impairment (389). Finally, it is difficult to ensure retrospectively that ALF and CLD patients received similar immunosuppressive regimes. However, during the period studied our unit had a single protocol that was rarely deviated from with CNI administration within 24 hours of transplantation. The similar post transplant CNI trough levels support this claim.

The findings of our study have important implications for patient management. Patients who undergo liver transplantation for ALF should not be considered a high risk group for developing CKD even when peri-operative acute renal impairment is severe. Consequently, we do not support the routine use of IL-2 receptor antagonists and delayed introduction of the CNI in this setting (295). Renal sparing immunosuppression such as mycophenolate and reduced dose tacrolimus could be considered in select patients, for example older females transplanted for non-paracetamol-induced ALF (297).

5.6 Conclusion

In conclusion, in this large single-centre study of patients transplanted for ALF we have shown that the severity of peri-operative renal dysfunction was not predictive of post transplant CKD. Despite greater peri-operative physiological derangement in ALF patients when compared with an age-sex-matched cohort transplanted for CLD renal function following transplantation was the same.

CHAPTER 6

The late decline in renal function after liver transplantation: Modifiable patient factors associated with the annualised change in eGFR from 6 months after transplantation

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6.1 Introduction

Chronic renal dysfunction is an important complication of liver transplantation, associated with significant morbidity and mortality (8,273,284). The 5 year cumulative incidence of severe CKD has been reported to be as high as 18%, and liver transplant recipients who develop CKD have a 5 times increased risk of death (8,282). The prevention of chronic renal dysfunction following liver transplantation has emerged as a priority for transplant physicians.

Early post-operative renal function is an indicator of renal outcome in this setting. Most patients demonstrate a steep decline in GFR during the first post-operative weeks with relative stabilisation thereafter, and 6- and 12-month post transplant GFR are consistent predictors of chronic renal dysfunction (8,264,275,276,277,278). Consequently, the peri-operative period has become a focus for interventions to lessen the decline in GFR. Cyclosporine and tacrolimus are considered to play a critical role in shaping renal function after transplantation (263,264,279,280). Several randomised controlled trials examining peri-operative minimisation of CNI exposure have been published (265,295,296). Yet, the results are mixed failing to demonstrate any convincing long term benefit in unselected patients.

The change in renal function beyond the initial post-operative period has received less attention. The steady state long-term rate of change in GFR allows individuals at risk of progression to CKD to be identified (282). Furthermore, by examining the relationship between rate of change and modifiable risk factors potential therapeutic

interventions can be suggested (282). In renal transplant recipients, patients who receive tacrolimus or non CNI based immunosuppression have a slower rate of decline in renal function compared to patients prescribed cyclosporine (436). In non-transplant CKD, hypertension, poor glycaemic control, smoking and possibly dyslipidaemia have been linked with a faster decline in GFR (282). Renal biopsies of liver transplant patients with chronic renal failure support a multi-factorial origin (274). However, the impact of such characteristics on the long-term rate of change in GFR and the development of renal dysfunction following liver transplantation remains unclear.

6.2 Aims

The aim of this study was to examine the long term change in GFR from 6 months following liver transplantation, and to identify modifiable factors associated with a faster rate of decline.

6.3 Methods

This was a retrospective single-centre case-note study of patients who underwent elective first liver transplantation between January 1st 1996 and December 31st 2000. The specified time period allowed the analysis of patients transplanted in an experienced centre with a follow-up time post transplant of greater than 5 years. This was an era when peri-operative renal sparing immunosuppression, and aggressive alteration of immunosuppression in response to moderate post transplant renal dysfunction, was infrequently practiced in our unit. Furthermore, cardiovascular risk factors were not as closely monitored. Therefore, variability of management between patients was less. To allow the analysis of change in renal function in a relatively homogeneous cohort of patients the records of the following groups were not reviewed: those listed for ALF (n=39) or joint liver/kidney transplantation (n=7), those who did not survive for 5 or more years post transplant (n=33) and those who changed CNI therapy during the follow-up period (n=9). Eighteen patients had incomplete data available and were also excluded from the analysis. The study group comprised a total of 97 patients.

Immunosuppression consisted of a CNI, azathioprine and prednisolone in most patients. Midway through the specified time period the unit policy for CNI changed from cyclosporine to tacrolimus. Prednisolone was usually discontinued by 3 to 6 months post transplant unless otherwise indicated. Deviation from the protocol occurred only in the setting of adverse event or graft rejection. Acute rejection was usually managed with 1g of methyl-prednisolone intravenously for 3 days followed

by re-introduction of oral steroids with or without increased dose of, or switch to, alternative CNI. Chronic rejection was managed with the latter and in 7 patients azathioprine was changed to mycophenolate.

Data was collected on the following at the time of listing for liver transplantation: age, gender, race, aetiology of liver disease, additional comorbidity, smoking status, MELD score, serum creatinine, serum sodium (hyponatraemia; serum sodium <135 mmol/l), presence of ascites and need for RRT. Post transplantation the following complications were documented only if occurring during the 5 year follow-up period: peri-operative RRT, number of acute rejection episodes, chronic rejection, disease recurrence and re-transplantation. The presence of diabetes, and diagnosed hypertension and dyslipidaemia were noted if present during the same time period. All blood pressures, body mass index (BMI) measurements, CNI trough levels and blood lipid concentrations (non-fasting) taken during routine outpatient appointments were recorded. These were averaged to provide the average systolic blood pressure, average diastolic blood pressure, average BMI, average cyclosporine trough level, average tacrolimus trough level, average cholesterol concentration and average triglyceride concentration. A comparable calcineurin trough level for the multivariate models was obtained for all patients regardless of CNI by expressing the trough as relative to the median value. Obesity was defined as an average BMI greater than or equal to 30.

GFR was estimated using the MDRD Study 6-variable equation (420).

$$\text{eGFR} = 170 \times \text{creatinine}(\text{mg/dl})^{-0.999} \times \text{age}^{-0.176} \times 1.180(\text{if black}) \times 0.762(\text{if female}) \times \text{serum urea nitrogen}^{-0.170} \times \text{albumin}^{0.138}$$

For each patient the eGFR was calculated from the serum creatinine level taken at the time of listing for liver transplantation (pre-transplant), and from routine outpatient clinics at 0.5, 1, 2, 3, 4 and 5 years post transplantation. As per the National Kidney Foundation CKD was defined as a sustained eGFR of less than 60 ml/min/1.73m²: stage 3, stage 4 and stage 5 CKD were defined as an eGFR of 30 to 59 ml/min/1.73m², 15 to 29 ml/min/1.73m², and less than 15 ml/min/1.73m² or on dialysis respectively (282).

Statistical analyses

Normally distributed continuous variables were compared using the Student's t-test and non-parametric continuous variables the Mann-Whitney test. For each patient the annualized change in eGFR was determined using simple linear regression of all eGFR measurements available from 6 months post transplantation (282). A negative annualised change in eGFR represents a decline in eGFR and a positive annualised change in eGFR represents an improvement in eGFR. The rate of change in eGFR expected with aging is 1 ml/min/1.73m² per year (282). Therefore, patients were described as having a decline in renal function greater than the rate expected with aging if the annualised change in eGFR was > -1.00 ml/min/1.73m²/year. Patients with an annualised change in eGFR of between -1.00 and +1.00 ml/min/1.73m²/year and those with an annualised increase in eGFR of > +1.00 ml/min/1.73m²/year were

considered to have unchanged renal function and improved renal function respectively (437).

Logistic regression analyses were used to examine the relationship between the annualised change in eGFR, and a decline in eGFR greater than the rate expected with aging, and the development of stage 3-5 CKD. Two separate multivariate models were constructed with all clinically relevant variables entered simultaneously. Pre-transplant hypertension was not included in the models because of small patient numbers. Multivariate linear regression analyses were then used to determine variables associated with the annualised change in eGFR. Again two multivariate models were constructed with all clinically relevant variables entered simultaneously. In model 2 the binary variables insulin dependent diabetes, diagnosed hypertension and diagnosed dyslipidaemia were replaced with the continuous variables average glucose, average systolic blood pressure and average cholesterol in an effort to examine whether modification of these factors might influence outcome. Age was not included in the models because of collinearity. Finally, logistic regression analysis was repeated to identify variables associated with a decline in renal function greater than the rate expected with aging. $p < 0.05$ was considered statistically significant at all times. Data was analysed using the SPSS 18 package.

The annualised change in eGFR is expressed as the mean with 95% confidence intervals (95% CI). All other values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

6.4 Results

Patient characteristics

Baseline characteristics of the patients at the time of listing for liver transplantation are outlined in Table 6.1. The median time from listing to transplantation was 39 (range 2-314) days.

Pre transplant renal function

Pre-transplant the mean eGFR of the entire cohort was 103 (SD 34) ml/min/1.73m² and the median serum creatinine was 81 (IQR 70-97) µmol/l. Three patients (3.1%) had an eGFR 30-59 ml/min/1.73m², 1 patient (1.0%) had an eGFR 15-29 ml/min/1.73m² and 0 patients had an eGFR <15 ml/min/1.73m² (Figure 6.1). Seventeen patients (17.5%) had refractory ascites and 23 (23.7%) had hyponatraemia.

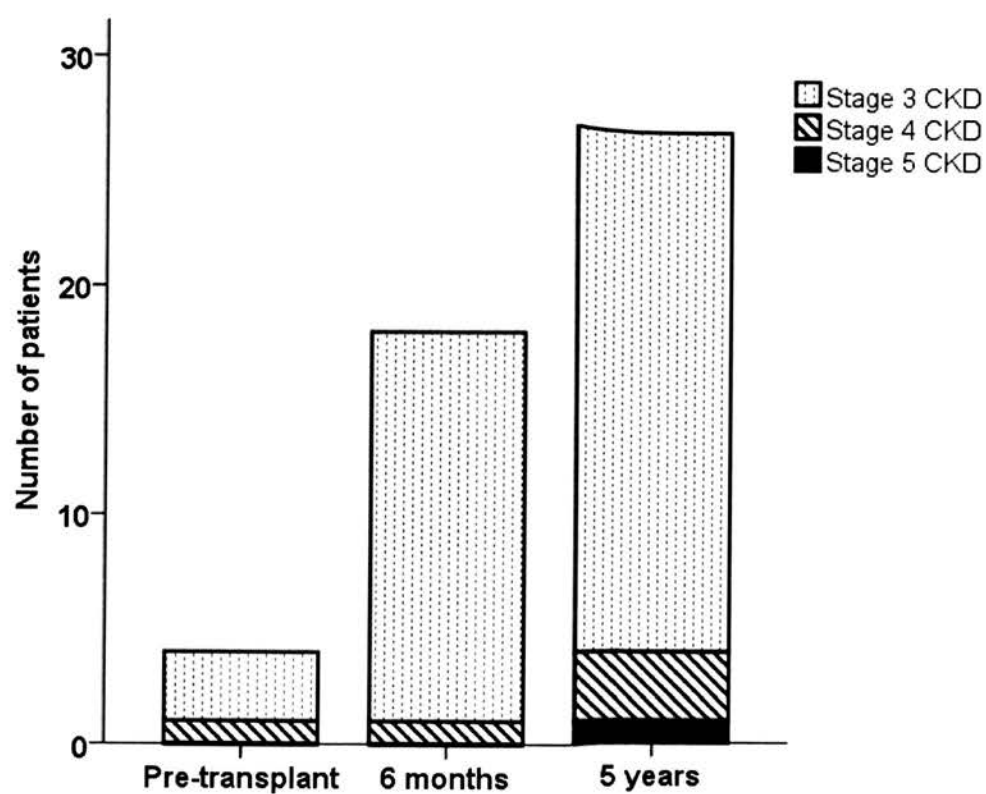
Table 6.1: Clinical characteristics of patients at time of listing for transplantation.

Clinical characteristics	
Age (years)	50.9 (10.3)
Male: Female	1:1.2
Caucasian	93 (95.9)
Liver disease aetiology:	
Primary biliary cirrhosis	35 (36.1)
Alcoholic liver disease	21 (21.6)
Sclerosing cholangitis	11 (11.3)
Cryptogenic cirrhosis	8 (8.2)
Hepatitis C	7 (7.2)
Autoimmune hepatitis	5 (5.1)
Hepatitis B	4 (4.1)
Other	6 (6.2)
Hepatocellular carcinoma	7 (7.2)
MELD score	16 (5)
Measure of renal function:	
Creatinine ($\mu\text{mol/l}$)	81 (70-97)
eGFR (ml/min/1.73m^2)	103 (34)
Sodium (mmol/l)	138 (135-140)
Hepatorenal syndrome	2 (2.1)
Renal replacement therapy	0
Ascites	59 (60.8)
Refractory ascites	17 (17.5)
Co-morbidity	
Diagnosed hypertension	6 (6.2)
Diagnosed dyslipidaemia	3 (3.1)
Diabetes mellitus	12 (12.4)
Insulin dependent diabetes	8 (8.2)
Active smoker	20 (20.6)
Obesity (wet weight)	18 (18.8)

Values expressed as mean (standard deviation), median (interquartile range) and number (percent) where appropriate.

Abbreviations: MELD, for end stage liver disease; eGFR, estimated glomerular filtration rate.

Figure 6.1: Prevalence of CKD pre-, and at 6-months and 5-years post liver transplantation.



Abbreviations: CKD, chronic kidney disease.

Change in renal function following transplantation

Eight patients (8.2%) required RRT during the immediate post-operative period.

By six months after transplantation the mean eGFR was 75 (SD 22) ml/min/1.73m² and the median serum creatinine was 103 (IQR 92-123) µmol/l. Seventeen patients (17.5%) had stage 3 CKD, 1 patient (1.0%) had stage 4 CKD and no patient had stage 5 CKD at this time point (Figure 6.1).

During subsequent years there was a progressive increase in the prevalence of renal dysfunction. At 5 years post transplant 23.7%, 3.1% and 1.0% of patients had stage 3, stage 4 and stage 5 CKD, respectively (Figure 1). The mean 5 year eGFR was 69 (SD 21) ml/min/1.73m² and the median 5 year serum creatinine was 110 (IQR 98-129) µmol/l.

eGFR declined at a mean rate of 1.08 ml/min/1.73m² per year (95% CI 2.13 to 0.03, p=0.045) from 6 months post transplant. Forty-seven patients (48.5%) demonstrated a decline in renal function greater than the rate expected with aging. Twenty patients (20.6%) had no change in renal function and 30 (30.9%) had an improvement in renal function. Assuming the mean annualised change in eGFR remained constant the estimated prevalence of stage 3, stage 4 and stage 5 CKD by 10 years post transplant was 29.9%, 6.2% and 6.2%, respectively.

Change in eGFR as a predictor of chronic kidney disease by 5 years post transplant

In patients who developed CKD the mean rate of decline in eGFR from 6 months post transplant was 2.50 (95% CI -4.03 to -0.97) ml/min/1.73m² per year compared with 0.52 (95% CI -1.35 to 0.30) ml/min/1.73m² per year for patients who did not (p=0.016). Sixty-seven percent of the CKD group demonstrated a decline in renal function greater than the rate expected with aging during the preceding years compared to only 41.4% of the non CKD group (p=0.026).

The annualised change in eGFR from 6 months after transplantation remained an independent predictor of 5 year CKD in a multivariate model including all clinically relevant variables simultaneously (table 6.2, p=0.001). Here, the more negative the change in eGFR (i.e. the greater the decline in eGFR) the greater the likelihood of CKD. In a similar multivariate model, a decline in renal function greater than the rate expected with aging was associated with a relative risk of CKD of 6.88 (95% CI 1.75-27.14, p=0.006, adjusted for age, gender, hepatitis C status, pre-transplant eGFR, refractory ascites and diabetes, peri-operative RRT, and CNI, data not shown).

Table 6.2: Univariate and multivariate logistic regression analyses of variables predictive of CKD by 5 years after liver transplantation.

	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (years)	1.07(1.02-1.12)	0.011	1.09 (1.00-1.18)	0.043
Female gender	7.67(2.40-24.52)	0.001	32.11 (3.00-343.71)	0.004
Hepatitis C	2.06 (0.43-9.90)	0.366	31.21 (1.41-688.86)	0.029
Pre-transplant				
eGFR (ml/min/1.73m ²)	0.97(0.95-0.99)	0.002	0.96 (0.93-0.99)	0.011
Refractory ascites	0.76 (0.23-2.59)	0.663	1.90 (0.21-17.32)	0.571
Diabetes mellitus	0.85 (0.21-3.40)	0.815	5.23 (0.30-91.05)	0.257
Peri-operative RRT	5.08 (1.12-22.98)	0.035	33.40 (3.63-307.05)	0.002
CNI: cyclosporine	1.99 (0.78-5.07)	0.151	2.13 (0.51-8.96)	0.302
ΔeGFR (ml/min/1.73m ² /year)	0.86 (0.76-0.98)	0.021	0.63 (0.47-0.84)	0.001

Reference group (relative risk 1.00): male gender, no hepatitis C, no refractory ascites, no diabetes mellitus, no peri-operative RRT, CNI: tacrolimus.

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor; ΔeGFR, mean annualised change in eGFR from 6 months to 5 years post transplant.

Factors associated with the change in renal function from 6 months after liver transplantation

Given the strong association between the decline in renal function from 6 months post transplant and the development of CKD statistical analyses were then performed to identify factors that may influence the long term change in eGFR. Clinical characteristics of the cohort during the follow-up period are outlined in table 6.3.

Table 6.3: Clinical characteristics of patients after liver transplantation.

Clinical characteristics	
Graft dysfunction:	
Early acute cellular rejection	33 (34.0)
Late acute cellular rejection	8 (8.2)
Chronic rejection	4 (4.1)
Re-transplant	6 (6.2)
Immunosuppression:	
CNI: cyclosporine	29 (29.9)
1 month cyclosporine trough	146 (50)
1 month tacrolimus trough	8.1 (3.0)
Co-morbidity:	
Diagnosed hypertension	46 (47.4)
Diagnosed dyslipidaemia	10 (10.3)
Diabetes mellitus	21 (21.6)
Insulin dependent diabetes	13 (13.4)
Obesity	33 (34.0)
Average value:	
Cyclosporine trough (mmol/l)	137 (20)
Tacrolimus trough (ug/l)	7.7 (1.3)
Systolic blood pressure (mmHg)	142 (15)
Diastolic blood pressure (mmHg)	82 (7.1)
Glucose (mmol/l)	5.8 (5.2-6.9)
Cholesterol (mmol/l, n=91)	5.0 (1.3)
Triglyceride (mmol/l, n=51)	1.6 (1.3-2.7)
Body mass index (BMI)	27.8 (5.1)

Values expressed as mean (standard deviation), median (interquartile range) and number (percent) where appropriate.

Abbreviations: CNI, calcineurin inhibitor.

Table 6.4: Multivariate linear regression analyses of variables associated with the annualised change in eGFR from 6 months post liver transplantation.

	Multivariate model 1			Multivariate model 2		
	B (95% CI)	β	P value	B (95% CI)	β	P value
6 month eGFR (ml/min/1.73m ²)	-0.108 (-0.141, -0.075)	-0.650	<0.001	-0.109 (-0.145, -0.073)	-0.680	<0.001
Female gender	-2.080 (-3.550, -0.609)	-0.284	0.006	-1.360 (-2.856, 0.137)	-0.192	0.074
Hepatitis C	-0.183 (-2.667, 2.300)	-0.013	0.884	-1.072 (-4.233, 2.089)	-0.069	0.502
Peri-operative RRT	-0.480 (-2.761, 1.800)	-0.036	0.676	-0.118 (-2.602, 2.366)	-0.009	0.925
CNI: cyclosporine	-0.637 (-2.033, 0.759)	-0.080	0.676	-0.820 (-2.273, 0.632)	-0.106	0.264
Average CNI trough	1.447 (-2.397, 5.291)	0.063	0.456	-0.149 (-4.324, 4.026)	-0.007	0.944
Insulin dependent diabetes	-0.559 (-2.484, 1.365)	-0.052	0.565			
Diagnosed hypertension	-1.523 (-2.788, -0.258)	-0.208	0.019			
Diagnosed dyslipidaemia	-2.300 (-4.420, -0.179)	-0.192	0.034			
Log average glucose (mmol/l)						
Average systolic blood pressure (mmHg)				-0.322 (-3.195, 2.531)	-0.025	0.818
Average cholesterol (mmol/l)				-0.076 (-0.128, -0.024)	-0.319	0.005
				-0.033 (-0.641, 0.574)	-0.012	0.913

Reference group (relative risk 1.00): male gender, no hepatitis C, no peri-operative RRT, CNI: tacrolimus, no insulin dependent diabetes, no diagnosed hypertension, no diagnosed dyslipidaemia.

Abbreviations: eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor

In a multivariate linear regression model adjusting for renal relevant variables, with the mean annualised change in eGFR as the dependent variable, a higher baseline eGFR ($p<0.001$), female gender ($p=0.006$), diagnosed hypertension ($p=0.019$) and diagnosed dyslipidaemia ($p=0.034$) were associated with a faster rate of decline in renal function (table 6.4, multivariate model 1). There was no association between the presence of hepatitis C infection, insulin dependent diabetes or immunosuppression, and change in eGFR. In a second multivariate model, in which binary variables were replaced with continuous, average systolic blood pressure was strongly associated with the change in renal function: for every 10 mmHg increase in average systolic blood pressure there was a $0.76 \text{ ml/min/1.73m}^2$ per year faster decline in eGFR ($p=0.005$, table 6.4, multivariate model 2).

Logistic regression analysis was then used to determine variables associated with a rate of decline in renal function greater than that expected with aging (Table 6.5). In this multivariate model after adjusting for all clinically relevant variables a higher 6 month eGFR ($p<0.001$), female gender ($p=0.024$) and diagnosed hypertension ($p=0.050$) were independent risk factors. There was a trend towards an association between cyclosporine and decline in renal function ($p=0.071$).

Table 6.5: Univariate and multivariate logistic regression analysis of variables associated with a rate of decline in renal function from 6 months post liver transplantation greater than the rate expected with aging.

	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
6 month eGFR (ml/min/1.73m ²)	1.03(1.01-1.06)	0.002	1.06 (1.03-1.10)	<0.001
Female gender	1.47 (0.66-3.29)	0.345	3.94 (1.20-12.95)	0.024
Hepatitis C	0.40 (0.07-2.17)	0.288	0.878 (0.11-7.27)	0.904
Peri-operative RRT	0.61 (0.14-2.72)	0.521	1.44 (0.23-8.84)	0.697
CNI: cyclosporine	3.37 (1.34-8.51)	0.010	2.68 (0.92-7.79)	0.071
Average CNI trough	0.832 (0.07-10.05)	0.885	1.10 (0.06-20.44)	0.949
Insulin dependent diabetes	0.89 (0.28-2.90)	0.859	0.86 (0.19-3.86)	0.842
Diagnosed hypertension	1.86 (0.83-4.16)	0.133	2.68 (1.00-7.19)	0.050
Diagnosed dyslipidaemia	1.68 (0.44-6.39)	0.444	2.95 (0.56-15.43)	0.200

Reference group (relative risk 1.00): male gender, no hepatitis C, no peri-operative RRT, CNI: tacrolimus, no insulin dependent diabetes, no diagnosed hypertension, no dyslipidaemia.

Abbreviations: eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor.

6.5 Discussion

In this single-centre study we have described for the first time the annualised change in eGFR following liver transplantation. In a homogeneous cohort of patients with long-term survival we examined the change in eGFR beyond the initial post-operative period, and current focus of CKD prevention. By estimating the steady state change in kidney function our aim was to identify possible modifiable risk factors for the progression to CKD. We have shown that liver transplant recipients had a clinically relevant decline in eGFR from 6 months post transplant. Our patients demonstrated a mean decline in eGFR of 1.1 ml/min/1.73m² per year, and almost half had a decline in renal function greater than the rate expected with aging. A decline in eGFR was a strong and independent predictor for the development of CKD. Multivariate modelling found a higher baseline eGFR, female gender, hypertension and dyslipidaemia to be associated with a faster rate of decline in renal function.

Several factors have been identified that may influence the development of chronic renal dysfunction following liver transplantation. Increasing age, white race, hepatitis C, pre-transplant diabetes mellitus, pre-transplant renal impairment and peri-operative acute renal failure are independent predictors of CKD (8,273,276,278,438). These variables highlight at the time of surgery the patients at increased risk of renal injury in whom preventative measures may be more crucial. However, few are potentially modifiable and little information has been gained regarding appropriate strategies to delay or avoid the progression to CKD in high risk individuals. In 2 out

of 3 large randomised controlled trials of unselected patients delayed peri-operative administration of tacrolimus did not impact on renal function by 1-year post transplant (265,295,296). All 3 studies were marred by failure to maintain low tacrolimus trough levels, and Boudjema et al's trial has confirmed that long-term reduced CNI exposure is probably more important (297). Reduction or withdrawal of CNI therapy once CKD has developed results in only a marginal improvement in kidney function (279,288,289,439,440,441). Therefore, prevention of CKD in liver transplant patients should be a priority (279).

The key outcome of our study was the observation that hypertension and dyslipidaemia were associated with the rate of decline in renal function after liver transplantation. Diagnosed hypertension was independently related to a faster decline in eGFR, and was a risk factor for a rate of decline greater than the rate expected with aging. Of course it is always difficult to disentangle the relationship between hypertension and renal function: high blood pressure can be a consequence as well as a cause of CKD (282). Even so, echoing findings in non-transplant patients with CKD and the general population, for every increment increase in average systolic blood pressure there was a faster rate of decline in GFR (282,442,443). This suggests that blood pressure control may be of particular importance in limiting the progression to CKD (459).

Diagnosed dyslipidaemia was also an independent predictor of change in eGFR, although we were unable to demonstrate a correlation with lipid levels. The diagnosis of dyslipidaemia was made by primary care or non-transplant hospital physicians,

and lipid levels were non-fasting and variably performed in the study cohort. Consequently, the reported lipid levels are probably a poor reflection of the true lipid profile. In the non-transplant setting the association between dyslipidaemia and a faster rate of progression of kidney disease is contentious (282). However, many studies in patients with and without renal dysfunction have linked high cholesterol, triglyceride and LDL levels, and low HDL levels, with the rate of change in GFR. Furthermore, lipid-lowering therapy has been shown to slow the decline in renal function (282,443,444).

In our study female patients had a faster rate of decline in eGFR than males, mirroring the gender difference demonstrated by renal transplant recipients (437). Female gender is a risk factor for chronic renal dysfunction following liver, heart and lung transplantation (8). In contrast, in non-transplant patients male gender is linked with a faster rate of decline in GFR (282). A possible explanation for this discrepancy is that females may be more susceptible to CNI mediated renal injury.

The strong association between baseline eGFR and change in eGFR may represent a statistical phenomenon. Nevertheless, we attempted to minimise 'regression to the mean' by estimating eGFR slope using a large number of measurements and by adjusting all analyses for baseline renal function (445,446). We speculate that patients who had a higher eGFR at 6 months post transplant had greater CNI exposure than those with renal dysfunction. Moreover, additional potential contributing factors such as hypertension and dyslipidaemia may have been monitored less closely in this group.

The retrospective nature of our study and relatively small patient numbers may explain the failure to demonstrate a relationship between diabetes, and immunosuppression, and change in eGFR (282). Furthermore, we did not have urate levels or urinary protein concentrations available on a sufficient number of patients to allow analysis (447). On the other hand, an advantage of the population size and methodology was that it allowed the close observation of risk factors over a prolonged time period. Additional potential limitations were the precision of the main outcome measures eGFR and eGFR slope. The former has been shown to be a less precise measure of GFR in liver transplant recipients than in other groups (419). However, it is the most widely accepted readily available measure of renal function. The precision of change in eGFR determined from the slope of eGFR over time increases with the duration of follow-up (445). All our patients had change in eGFR calculated from six eGFR measurements and a prolonged follow-up time of five years.

6.6 Conclusion

In conclusion, our study has shown for the first time that liver transplant recipients have a clinically relevant decline in eGFR from 6 months following transplantation. We have identified modifiable factors that may influence the change in eGFR and increase the risk of progression to CKD. Our study emphasises the multi-factorial nature of renal dysfunction following liver transplantation. Prospective studies are required to examine the effects of aggressive blood pressure and lipid control on the development of CKD in liver transplant patients.

CHAPTER 7

Renal dysfunction in liver disease: General discussion

Renal dysfunction is common and continues to have devastating consequences in patients with all aspects of liver disease. In cirrhosis and ALF, management remains primarily supportive and does not impact on prognosis. Only liver transplantation offers survival benefit, but is a finite resource. Moreover, liver transplantation itself is complicated by both AKI and CKD.

The studies presented in this thesis have helped to further our knowledge of portal hypertension-related renal dysfunction, AKI in ALF and CKD after liver transplantation. By doing so, we have moved one step closer to improving patient morbidity and mortality as a result of renal dysfunction in liver disease.

7.1 Renal dysfunction in cirrhosis

Hypothesis: In patients with cirrhosis increased activity of endogenous endothelin-1 is involved in the pathophysiology of renal dysfunction through renal vasoconstriction, and endothelin-1 antagonism will reverse these effects (Chapter 2).

ET-1 has been implicated in the pathophysiology of portal hypertension-related renal dysfunction. In this randomised, double-blind, placebo controlled, crossover study of patients with advanced cirrhosis and refractory ascites, acute combined ET-A and ET-B receptor blockade caused a fall in GFR despite no change in systemic haemodynamics or total renal blood flow, and a marked reduction in UFR. Selective ET-A receptor antagonism had no haemodynamic or renal tubular effects suggesting

that the ET-B receptor plays a key role in this setting. These findings are consistent with a reno-protective role for ET-1 in portal hypertension-related renal dysfunction.

7.2 Renal dysfunction in acute liver failure

Hypothesis: In acute liver failure the systemic inflammatory response syndrome is associated with the development of acute kidney injury (Chapter 3).

Current hypothesis suggests that the renal dysfunction of ALF and the renal dysfunction of portal hypertension share similar pathophysiology. However, ALF has distinct clinical characteristics and the circulatory derangement may be more comparable with sepsis. In a retrospective study we demonstrated for the first time that in ALF renal dysfunction is associated with SIRS. On univariate analysis patients with AKI were more likely to be hypothermic, had a faster heart rate, a higher WCC and a lower PaCO₂. Multivariate analysis confirmed that SIRS was a risk factor for the development of AKI. Importantly, this association was independent of the presence of infection and of severity of liver injury as assessed by the KCH prognostic criteria. We propose that the systemic inflammatory cascade plays a key role in its pathogenesis.

7.3 Renal dysfunction as a prognostic indicator in liver disease

Hypothesis: Estimated glomerular filtration rate is superior to serum creatinine in predicting prognosis in patients on the liver transplant waiting list (Chapter 4).

In cirrhosis the spectrum of renal dysfunction evolves in parallel with advancing disease. Serum creatinine as a continuous variable is an independent predictor of mortality in those on the liver transplant waiting-list, and is a component of the MELD score. However, creatinine is influenced by age, gender and race, and in this role may disadvantage some individuals. The MDRD eGFR takes into account these variables and may be a superior measure of renal function. In this study we demonstrated that listing eGFR was comparable, but not superior, to listing serum creatinine for prediction of 3-month waiting-list mortality, and when substituted for serum creatinine eGFR did not improve the prognostic accuracy of MELD. Our findings support the need for further research to identify more precise non creatinine based measures of renal function in cirrhosis.

7.4 Implications of renal dysfunction for post liver transplant renal function

Hypothesis: Given potentially different pathophysiological mechanisms underlying renal dysfunction, patients transplanted for acute liver failure have

comparatively better long term renal outcomes than patients transplanted for chronic liver disease (Chapter 5).

Following on from Chapter 3, we further explored our hypothesis that the renal dysfunction of ALF and the renal dysfunction of CLD have distinct pathophysiological mechanisms. In patients super-urgently transplanted for ALF we found that peri-operative AKI did not appear to have the same long-lasting consequences for renal function as demonstrated by patients undergoing elective liver transplantation. Contrary to observations in CLD, pre-transplant AKI and RRT were not associated with CKD. Despite marked differences in the peri-operative clinical condition of patients transplanted for ALF and an age-sex-matched cohort transplanted for CLD long term renal outcomes were the same. Our results support an alternative pathophysiological process underlying the renal injury that occurs in ALF.

7.5 The late decline in renal function after liver transplantation

Hypothesis: Modifiable patient factors are associated with the long term decline in renal function after liver transplantation (Chapter 6).

Strategies to delay or avoid the long-term decline in renal function and progression to CKD in liver transplant recipients remain unclear. In this final study, we observed for the first time that liver transplant recipients have a clinically relevant decline in eGFR from 6 months following transplantation, beyond the initial post-operative

period and current focus of chronic kidney disease prevention. Our patients demonstrated a mean decline in eGFR of 1.1 ml/min/1.73m² per year, and almost half had a decline in renal function greater than the rate expected with aging. A decline in eGFR was a strong and independent predictor for the development of CKD. Multivariate modelling found a higher baseline eGFR, female gender, hypertension and dyslipidaemia to be associated with a faster rate of decline in renal function emphasising the multi-factorial nature of renal dysfunction after liver transplantation.

CHAPTER 8

Future directions

8.1 Future directions specific to this thesis

To expand on the presented results and take this thesis forward several important studies should be performed.

Firstly, the findings in the pilot study of ET-1 receptor antagonism in advanced CLD described in Chapter 2 require confirmation in a larger cohort of patients. A forearm study as performed by Helmy but in patients with refractory ascites would elucidate the relative contributions of the ET-A and ET-B receptors, and perhaps confirm that the ET-B receptor plays a more prominent role in later disease (361,363). The most striking renal effect we observed was the dramatic fall in UFR with combined ET-A and ET-B blockade. We have stored plasma and urine samples that we are soon to analyse to determine whether this reflected inhibition of natriuresis or free water clearance. It would be interesting to compare findings with ET-1 receptor antagonism in patients with diuretic controlled ascites.

Secondly, the studies in Chapters 3 and 5 highlight for the first time the likely differing pathophysiological mechanisms underlying the renal dysfunction of ALF. The next step in pursuing our hypothesis is to perform a prospective observational study in patients with acute liver injury and ALF to describe the association between the evolution in the systemic inflammatory response and haemodynamic renal and tubular dysfunction. In the first instance we intend to perform serial blood sampling (for DAMPs, cytokines and other inflammatory mediators), systemic and renal

haemodynamic monitoring and urine sampling (for markers of tubular injury and function), and to correlate them with clinical outcomes.

8.2 Summary of ongoing work

During the process of this work I acquired an understanding of renal dysfunction in liver disease that has allowed me to develop unique hypotheses in as yet understudied renal processes. As a direct result of these skills and knowledge I am now a Clinical Lecturer in Hepatology in the Queen Elizabeth Hospital Birmingham and am developing a programme of research in this field.

My main focus of interest is AKI during the immediate post operative period after liver transplantation. AKI is a major cause of morbidity and mortality in this setting. Aetiology is multi-factorial with most studies concentrating on recipient risk factors such as pretransplant renal function, and immunosuppression. However, there is mounting evidence to suggest that liver graft injury at the time of transplantation, by driving a systemic inflammatory response, may play an important role in modifying both short and long term renal outcomes. It follows that the increased use of extended criteria grafts may have negative consequences for post transplant renal function. Furthermore, strategies targeting graft injury may minimise the peri-operative renal 'hit' and be beneficial for patient outcomes.

I have observed that AKI is more frequent in donation after cardiac death liver transplant recipients, and in these patients peak peri-operative aspartate aminotransferase, a surrogate marker of hepatic ischaemia reperfusion injury, is the only variable associated with renal dysfunction (448). Similarly, hepatic ischaemia reperfusion injury demonstrates a strong relationship with AKI in donation after

brain death recipients, and the optimal graft quality of split livers is associated with a reduced frequency of RRT (449,450). More recently, I have confirmed that the increasing use of extended criteria donors has been associated with an increased incidence of AKI despite better preoperative optimisation of recipient renal function and less aggressive immunosuppression (451). Increased donor age, increased donor BMI, higher donor serum sodium and increasing warm ischaemic time are independently associated with the development of AKI (451).

My future intention is to characterise the peri-operative evolution of the systemic inflammatory response in patients undergoing liver transplantation with higher risk grafts, and to delineate the relationship with haemodynamic and renal derangement.

8.3 Clinical perspective

We are in an era of increasing organ shortage. I hypothesise that hepatic ischaemia reperfusion injury and the secondary systemic inflammatory response may play a critical role in the pathogenesis of AKI after liver transplantation. I believe that my work is the first step in identifying modifiable disease processes that ultimately may lead to the discovery of interventions that influence the patient morbidity and mortality.

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APPENDIX

Oral presentations arising directly from this thesis

1. Leithead JA, Ferguson JW, Hayes PC. Chronic renal failure post liver transplantation; prevalence and rate of decline. Scottish Society of Gastroenterology Meeting 2006.
2. Leithead JA, Ferguson JW, Hayes PC. Estimated glomerular filtration rate may be superior to creatinine in predicting prognosis on the waiting list for liver transplantation. Scottish Society of Gastroenterology Meeting 2008.

Poster presentations arising directly from this thesis

1. Leithead JA, Ferguson JW, Hayes PC. Chronic renal failure post liver transplantation; prevalence and rate of decline. British Society of Gastroenterology Meeting 2007, European Association for the Study of the Liver Meeting 2007.
2. Leithead JA, Ferguson JW, Bates CM, Davidson JS, Simpson KJ, Hayes PC. Chronic kidney disease following liver transplantation for acute liver failure. British Association for the Study of the Liver Meeting 2007, European Association for the Study of the Liver Meeting 2008.
3. Leithead JA, Ferguson JW, Hayes PC. Estimated glomerular filtration rate is a predictor of death on the waiting list for liver transplantation. British Society of Gastroenterology Meeting 2008, European Association for the Study of the Liver Meeting 2008.
4. Leithead JA, Ferguson JW, Bates CM, Davidson JS, Bathgate A, Hayes PC, Simpson KJ. The systemic inflammatory response to acute liver failure predicts the development of renal failure. British Society of Gastroenterology Meeting 2008, European Association for the Study of the Liver Meeting 2008.
5. Leithead JA, Ferguson JW, Hayes PC. Preventing the progression to chronic kidney disease post liver transplantation. European Association for the Study of the Liver Meeting 2008.
6. Leithead JA, Ferguson JW, MacKenzie SM, Hayes PC. Measured creatinine clearance is superior to serum creatinine as a prognostic indicator in patients on the liver transplant waiting-list. American Association for the Study of Liver Diseases Meeting 2008.

Publications arising directly from this thesis

1. Leithead JA, Ferguson JW, Bates CM, Davidson JS, Lee A, Bathgate AJ, Hayes PC, Simpson KJ. The systemic inflammatory response syndrome is predictive of renal dysfunction in patients with non-paracetamol-induced acute liver failure. *Gut* 2009;58(3):443-9. [Appended]
2. Leithead JA, MacKenzie SM, Ferguson JW, Hayes PC. Is estimated glomerular filtration rate superior to serum creatinine in predicting prognosis on the waiting list for liver transplantation? *Transpl Int* 2011;24:482-488. [Appended]
3. Leithead JA, Ferguson JW, Bates CM, Davidson JS, Simpson KJ, Hayes PC. Chronic kidney disease after liver transplantation for acute liver failure is not associated with peri-operative renal dysfunction. *Am J Transpl* 2011;11(9):1905-1915. [Appended]
4. Leithead JA, Ferguson JW, Hayes PC. Modifiable patient factors are associated with the late decline in renal function following liver transplantation. *Clin Transpl* 2012; 26(3):E316-23. [Appended]

The systemic inflammatory response syndrome is predictive of renal dysfunction in patients with non-paracetamol-induced acute liver failure

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ABSTRACT

Background: Although renal dysfunction is a common complication of acute liver failure (ALF) with significant prognostic implications, the pathophysiological mechanisms remain unclear. The current hypothesis suggests that the renal dysfunction may mirror the hepatorenal syndrome of cirrhosis. However, ALF has distinct clinical characteristics and the circulatory derangement may be more comparable with sepsis.

Objectives: To examine the relationship between the systemic inflammatory response syndrome (SIRS) and renal dysfunction in ALF, and to identify additional risk factors for renal dysfunction.

Methods: A single-centre retrospective study of 308 patients with ALF was carried out. Renal dysfunction was defined according to the RIFLE criteria for acute kidney injury.

Results: 67% of patients developed renal dysfunction. On univariate analysis, renal dysfunction patients were more likely to be hypothermic ($p = 0.010$), had a faster heart rate ($p < 0.001$), a higher white cell count ($p = 0.001$) and a lower PaCO_2 ($p = 0.033$). 78% of renal dysfunction patients and 53% of non-renal dysfunction patients had SIRS ($p < 0.001$). On multivariate analysis, the risk factors for renal dysfunction were age ($p = 0.024$), fulfilled Kings College Hospital prognostic criteria ($p < 0.001$), hypotension ($p < 0.001$), paracetamol-induced ALF ($p < 0.001$), infection ($p = 0.077$) and SIRS ($p = 0.017$). SIRS remained an independent predictor of renal dysfunction in the subgroup of patients with non-paracetamol-induced ALF ($n = 91$, $p = 0.001$). In contrast, in patients with paracetamol-induced ALF ($n = 217$), no relationship between SIRS and renal dysfunction was demonstrated ($p = 0.373$).

Conclusion: SIRS is strongly associated with the development of renal dysfunction in patients with non-paracetamol-induced ALF. It is proposed that the systemic inflammatory cascade plays a key role in its pathogenesis.

Renal failure is a common complication of acute liver failure (ALF) that occurs in 43% of patients with grade IV hepatic encephalopathy.¹ It is associated with increased mortality, emphasised by the inclusion of serum creatinine in the Kings College Hospital (KCH) prognostic criteria.^{2,3} Despite the clinical burden of renal dysfunction in this setting, the aetiological mechanisms and risk factors remain unclear. Consequently, treatment options without liver transplantation are limited and potential prophylactic measures are unknown.

The current hypothesis suggests that the renal dysfunction of ALF and hepatorenal syndrome of cirrhosis share similar pathophysiology.^{4,5} Supporting this argument, the circulatory dysfunction of ALF, which is characterised by reduced systemic vascular resistance and high cardiac output, appears to parallel that of chronic liver disease.⁶⁻⁹ The marked reduction in renal blood flow and glomerular filtration rate that is associated with renal failure mirrors the renal perfusion changes of advanced cirrhosis.⁷ However, ALF has distinct haemodynamic features that indicate that the renal dysfunction of ALF and cirrhosis may not be comparable. First, clinically significant portal hypertension is not always present in patients with ALF and renal dysfunction.⁹ Moreover, the degree of portal hypertension rarely equates with that of hepatorenal syndrome in cirrhosis: the mean reported hepatic venous pressure gradient for patients with ALF and renal dysfunction is 14 mm Hg compared with 21 mm Hg for patients with hepatorenal syndrome.⁹⁻¹¹ Secondly, animal studies imply that the systemic vasodilatation may be more generalised and not limited to the splanchnic circulation as occurs in cirrhosis.¹²⁻¹⁵ Pigs with ALF demonstrate reduced hind leg and renal vascular resistance, which contrasts with the femoral and renal vasoconstriction of hepatorenal syndrome.¹²⁻¹⁵ Vasodilatation within these vascular beds is more in keeping with the hyperdynamic syndrome of sepsis than of advanced cirrhosis.^{16,17} Recently it had been demonstrated that the systemic inflammatory response syndrome (SIRS) is often present in patients with ALF.^{18,19} SIRS is associated with progression of hepatic encephalopathy, suggesting that the systemic inflammatory response may be involved in its pathogenesis.^{18,19} Following on from this, we postulate that the systemic inflammatory response may play a role in the pathogenesis of renal dysfunction in these patients.

Additional factors that may contribute to renal dysfunction in ALF include hypovolaemia, nephrotoxic drugs, infection and disseminated intravascular coagulation.^{1,20-22} Paracetamol has been shown in animal models to have a direct nephrotoxic effect, and in humans there are case reports of renal failure following paracetamol overdose in the absence of significant hepatic injury.²³⁻²⁵ Nevertheless, the frequency of renal dysfunction in patients with paracetamol-induced ALF has not been shown to be higher than in other aetiologies.¹

At present there is a poor understanding of the pathogenesis and risk factors for renal dysfunction

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in ALF. The primary aim of our study was to examine whether SIRS is associated with renal dysfunction in a large cohort of patients with ALF. Our secondary aim was to identify additional risk factors for the development of renal dysfunction in ALF.

PATIENTS AND METHODS

This was a retrospective study of 442 patients admitted to a single tertiary referral centre with ALF between November 1992 and June 2007. One hundred and seven patients who were ventilated prior to admission were excluded from the analysis because of the influence of sedation and mechanical ventilation on SIRS.^{18–19} A further 6 patients who received renal replacement therapy but did not fulfil the definition for renal dysfunction, and 21 patients who did not have a peak serum creatinine available, were also not assessed. Therefore, the study cohort comprised 308 patients.

The mean age was 39.7 (SD 14.7) years and the male to female ratio was 1:1.3. The causes of ALF were paracetamol overdose (217 patients), seronegative hepatitis (39 patients), idiosyncratic drug reaction (23 patients), hepatitis B (5 patients), autoimmune (5 patients), alcoholic hepatitis (5 patients), Budd–Chiari (5 patients), acute fatty liver of pregnancy (3 patients), hepatitis A (2 patients), Wilson disease (2 patients) and non-paracetamol drug overdose (2 patients). One hundred and twelve patients died, 112 survived and 83 underwent liver transplantation.

ALF was defined as severe liver injury with hepatic encephalopathy in which the onset of encephalopathy was within 8 weeks of the first symptoms of illness, and in the absence of pre-existing liver disease.²⁶ A patient was considered to have significant renal dysfunction if they fulfilled the RIFLE criteria (acronym indicating Risk of renal dysfunction, Injury to the kidney, Failure of kidney function, Loss of kidney function and End-stage renal disease) for acute kidney injury (AKI): peak serum creatinine ≥ 2 times the baseline level.²⁷ The baseline serum creatinine was unavailable and therefore was estimated from the Modification of Diet in Renal Disease formula with an assumed glomerular filtration rate at the lower end of normal (75 ml/min/1.73 m²), as outlined by the Acute Dialysis Quality Initiative (ADQI) workgroup.²⁷ Serum creatinine may theoretically overestimate the glomerular filtration rate in patients with ALF. Therefore, we chose the criteria for AKI rather than acute kidney failure to allow detection of significant renal dysfunction with greater sensitivity.²⁷

Data were collected prospectively and entered into a dedicated database. The following variables were recorded at the time of hospital admission: temperature, pulse, white cell count, neutrophil count, platelet count, international normalised ratio (INR), serum electrolytes, serum bilirubin, alanine aminotransferase (ALT), albumin, arterial hydrogen ion (H⁺), bicarbonate (HCO₃⁻), PaCO₂ and lactate. Peak creatinine and peak INR were documented. SIRS was defined as two or more of temperature $<36^{\circ}\text{C}$ or $>38^{\circ}\text{C}$, heart rate >90 beats/min (bpm), white cell count $<4 \times 10^9/\text{l}$ or $>12 \times 10^9/\text{l}$ and PaCO₂ <4.3 kPa.²⁸ Regular alcohol intake prior to admission was recorded; alcohol excess was defined for women as >112 g/week and for men as >168 g/week as per the UK guidelines.²⁹ The presence of the following variables at any point during admission was documented: ventilation, treatment for increased intracranial pressure (ICP), hypotension (systolic blood pressure <90 mm Hg), need for inotropes (noradrenaline/adrenaline), hypoglycaemia, prognosis assessed using the KCH criteria, and infection (positive cultures and/or ascitic fluid

polymorphonuclear count $>250/\text{mm}^3$ and/or radiological evidence of infection).³ All patients had cultures (sputum/stool/ascites/intravascular catheter where appropriate) and a chest x ray performed routinely on admission, and repeat cultures if clinically indicated. Spontaneous survival and death were defined as survival without liver transplantation and death without liver transplantation, respectively: patients who received a liver transplant were excluded from all survival analyses.

Statistical analyses

Normally distributed continuous variables and non-parametric continuous variables were compared using the Student t test and Mann–Whitney test, respectively. χ^2 analysis was used for comparison of categorical data. Stepwise backwards logistic regression models, verified with forwards models, were used to determine the factors independently associated with death and AKI. Patients who received a liver transplant were excluded from all survival analyses. Only those variables with $p < 0.10$ were included in the multivariate analyses. $p < 0.05$ was considered significant. Data was analysed using the SPSS 15 package.

All values are expressed as mean (SD) and median and interquartile range (IQR) as appropriate.

RESULTS

Prevalence of AKI

At the time of admission to hospital, the median serum creatinine was 149 (IQR 96–256) $\mu\text{mol/l}$, and 133 patients (43%) fulfilled the criteria for AKI. Thereafter, most patients demonstrated a decline in renal function: 196 patients (64%) had a $\geq 10\%$ increase in serum creatinine, 106 patients (34%) had no change in serum creatinine and 6 patients (2%) had a $\geq 10\%$ improvement in serum creatinine. The median peak serum creatinine was 288 (IQR 135–392) $\mu\text{mol/l}$. Two hundred and eight patients (67%) fulfilled the criteria for AKI at some point during their illness, of whom 70% underwent renal replacement therapy.

Prevalence of SIRS

Seventy percent of patients had SIRS. SIRS was more prevalent in patients with paracetamol-induced ALF compared with patients with non-paracetamol-induced ALF (77% vs 54%, $p < 0.001$). The frequency of SIRS was not affected by the presence of infection (infected, 70%; non-infected, 70%; $p = 0.880$). SIRS was not more common in patients who achieved KCH poor prognostic criteria (achieved, 72%; not achieved, 67%; $p = 0.344$), although a greater proportion of patients who were hypoglycaemic (hypoglycaemic, 78%; non-hypoglycaemic, 65%; $p = 0.040$) or required treatment for increased ICP (treatment, 79%; no treatment, 66%; $p = 0.032$) demonstrated SIRS.

AKI, SIRS and mortality

Patients with AKI had a prolonged hospital admission (AKI, 11 (7–20) days; non-AKI, 6 (4–10) days, median (IQR); $p < 0.001$) and reduced spontaneous survival (AKI, 36%; non-AKI, 84%; $p < 0.001$, fig 1). There was no relationship between AKI and liver transplantation (AKI, 25%; non-AKI, 33%; $p = 0.118$). However, the transplanted patients who had AKI preoperatively had a longer postoperative hospital stay (AKI, 26 (20–33) days; non-AKI, 15 (13–20) days, median (IQR); $p < 0.001$) and a

trend towards reduced survival to hospital discharge (AKI, 67%; non-AKI, 85%; $p = 0.064$).

The presence of SIRS was also associated with reduced spontaneous survival (SIRS, 42%; non-SIRS, 60%, $p = 0.035$). Nevertheless, a similar proportion of patients with and without SIRS received a liver transplant (26% vs 32%, $p = 0.386$) and there was no association between SIRS and survival following transplantation (SIRS, 70%; non-SIRS, 71%, $p = 0.957$).

Other variables associated with spontaneous survival are outlined in table 1. On multivariate analysis, AKI, and not SIRS, was independently associated with mortality.

Relationship between AKI and the aetiology of ALF

Patients who developed AKI were more likely to have ALF as a result of paracetamol ingestion than those who did not have AKI (80% vs 51%, $p < 0.001$). The median peak serum creatinine for patients with paracetamol-induced ALF was 300 (IQR 183–413) $\mu\text{mol/l}$ and for patients with non-paracetamol-induced ALF it was 149 (IQR 99–322) $\mu\text{mol/l}$ ($p < 0.001$). Seventy-six percent of patients with paracetamol-induced ALF and 46% of patients with non-paracetamol-induced ALF fulfilled the criteria for AKI ($p < 0.001$).

Relationship between AKI and the severity of ALF

Clinical and biochemical characteristics of the AKI and non-AKI patients on admission to hospital are outlined in table 2. Patients with AKI had a higher ALT level ($p < 0.001$), INR ($p = 0.001$) and lactate ($p = 0.007$), and were more likely to be acidotic (31% vs 0%, $p < 0.001$).

By definition all patients became encephalopathic. However, the AKI group were more likely than the non-AKI group to be ventilated (77% vs 56%, $p < 0.001$) and a greater proportion required treatment for increased ICP (AKI, 34%; non-AKI, 14%; $p < 0.001$). In addition, patients with AKI were more likely to

demonstrate hypoglycaemia (AKI, 50%; non-AKI, 18%; $p < 0.001$) and to fulfil KCH poor prognostic criteria (AKI, 64%; non-AKI, 37%; $p < 0.001$).

Relationship between AKI and SIRS

Patients who developed AKI had evidence of a greater systemic inflammatory response. At the time of hospital admission they were more likely to have a temperature $< 36^\circ\text{C}$ (AKI, 34%; non-AKI, 12%; $p = 0.010$), had a faster heart rate ($p < 0.001$), a higher white cell count ($p = 0.001$) and a lower PaCO_2 ($p = 0.033$) (table 2). Furthermore, a greater proportion of those with AKI developed hypotension (AKI, 63%; non-AKI, 13%; $p < 0.001$) and required inotropic support (AKI, 56%; non-AKI, 9%; $p < 0.001$).

Seventy-eight percent of the AKI patients had SIRS compared with 53% of the non-AKI patients ($p < 0.001$). An increasing number of components of SIRS was associated with an increased probability of renal dysfunction: 47, 60, 69, 79 and 81% of the patients with 0, 1, 2, 3 and 4 components of SIRS at admission, respectively, developed AKI ($p = 0.047$). The AKI group were more likely to demonstrate infection (AKI, 56%; non-AKI, 38%; $p = 0.003$). Nevertheless, in both those with infection (AKI, 74%; non-AKI, 57%; $p = 0.062$) and those without infection (AKI, 82%; non-AKI, 50%; $p < 0.001$) SIRS was more common in the AKI patients.

Independent risk factors for the development of AKI in ALF

A multivariate analysis was performed to identify the factors that are independently associated with renal dysfunction (table 3). This revealed that the severity of ALF, the systemic inflammatory response to ALF and superimposed factors may all be relevant. The variables independently associated with AKI were age ($p = 0.024$), fulfilled KCH poor prognostic criteria ($p < 0.001$), hypotension ($p < 0.001$), SIRS ($p = 0.017$), superimposed infection ($p = 0.077$) and paracetamol as the cause of ALF ($p < 0.001$).

In view of the strong association of paracetamol with AKI, the entire cohort was then subdivided into two groups: paracetamol-induced ALF and non-paracetamol-induced ALF based on the presence or absence of paracetamol-induced liver injury, respectively. The associations between the severity of ALF and AKI, and SIRS and AKI, were reassessed.

AKI in paracetamol-induced ALF

In the paracetamol-induced ALF subgroup, patients with AKI had evidence of more severe liver injury when compared with patients with no AKI (table 4): they were more likely to be hypoglycaemic ($p < 0.001$), to require treatment for increased ICP ($p = 0.003$) and to fulfil KCH poor prognostic criteria ($p < 0.001$). In contrast, the AKI patients were not more likely to demonstrate SIRS ($p = 0.373$) and were not more likely to have infection ($p = 0.287$). However, they did have a lower admission temperature ($p < 0.001$), higher white cell count ($p = 0.043$), trend towards a lower PaCO_2 ($p = 0.070$), and were more likely to have ≥ 3 systemic inflammatory response components (AKI, 43%; non-AKI, 27%; $p = 0.055$). On multivariate analysis the factors associated with AKI in patients with paracetamol-induced ALF were age (OR 1.03; 95% CI 1.00 to 1.06, $p = 0.059$), treatment for increased ICP (OR 2.74; 95% CI 1.05 to 7.15, $p = 0.039$) and hypotension (OR 11.38; 95% CI 4.19 to 30.91, $p < 0.001$).

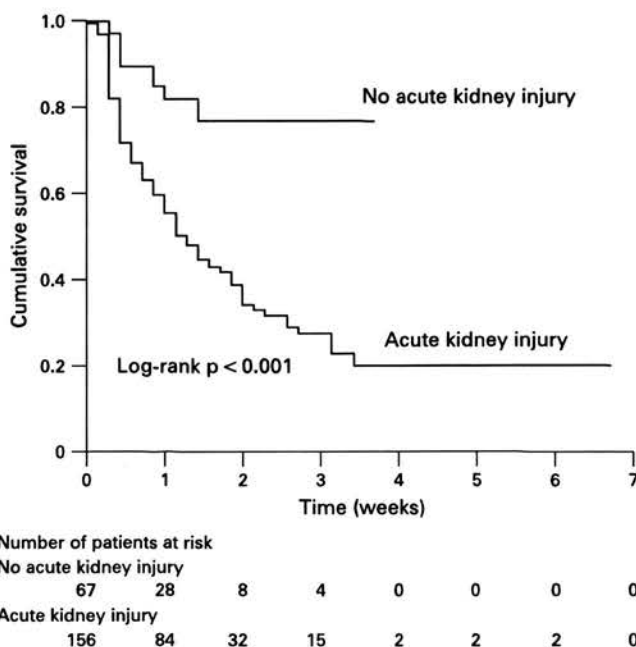


Figure 1 Spontaneous survival in patients with acute liver failure subdivided based on the presence or absence of acute kidney injury. Patients who received a liver transplant were excluded from the survival analysis.

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Table 1 Factors predictive of mortality on univariate and multivariate analysis in patients with ALF*

Variable	Univariate OR (95% CI)	p Value	Multivariate OR (95% CI)	p Value
Age (years)	1.04 (1.02 to 1.06)	<0.001	1.06 (1.03 to 1.09)	<0.001
Hypoglycaemia	6.20 (3.40 to 11.27)	<0.001	4.97 (2.27 to 10.91)	<0.001
Treatment for increased ICP	5.17 (2.54 to 10.55)	<0.001	6.72 (2.56 to 17.67)	<0.001
SIRS	2.00 (1.04 to 3.85)	0.037		
AKI	9.18 (4.45 to 18.94)	<0.001	5.48 (2.20 to 13.64)	<0.001

Reference group (relative risk 1.00): no hypoglycaemia, no treatment for increased ICP, no SIRS, no acute kidney injury, male gender, non-paracetamol-induced ALF, no infection.

*Variables not associated with mortality: female gender ($p = 0.502$), paracetamol-induced ALF ($p = 0.129$), infection ($p = 0.547$).

AKI, acute kidney injury; ALF, acute liver failure; ICP, intracranial pressure; SIRS, systemic inflammatory response syndrome.

AKI in non-paracetamol-induced ALF

In the non-paracetamol-induced ALF subgroup, patients with AKI did not have evidence of more severe liver injury relative to patients with no AKI (table 4). Despite a trend towards an association between AKI and hypoglycaemia ($p = 0.062$), patients with renal dysfunction were not more likely to require treatment for increased ICP ($p = 0.285$) or to achieve KCH poor prognostic criteria ($p = 0.491$). Nevertheless, there was a strong relationship between AKI and SIRS ($p < 0.001$, fig 2). Patients with AKI were more likely to have infection ($p = 0.002$), and in both infected (AKI, 78%; non-AKI, 40%; $p = 0.017$) and non-infected patients (AKI, 73%; non-AKI, 33%; $p = 0.037$) the prevalence of SIRS was greater in those with AKI. On multivariate analysis the factors associated with AKI in patients with non-paracetamol-induced ALF were hypotension (OR

8.63; 95% CI 2.63 to 28.3, $p < 0.001$) and SIRS (OR 6.98; 95% CI 2.13 to 22.83, $p = 0.001$).

DISCUSSION

We have shown for the first time that in ALF renal dysfunction is associated with SIRS. On univariate analysis, patients with AKI were more likely to be hypothermic, had a faster heart rate, a higher white cell count and a lower PaCO_2 . Multivariate analysis confirmed that SIRS was a risk factor for the development of AKI. Importantly, this association was independent of the presence of infection and of severity of liver injury as assessed by the KCH prognostic criteria. A further novel finding of our study is the strikingly increased risk of AKI demonstrated by patients with paracetamol-induced ALF. This relationship supports in vitro animal data and clinical suspicion that paracetamol has a direct nephrotoxic effect. Drug-induced renal injury could mask any relationship between SIRS and renal dysfunction. Therefore, a key finding of our study is the strong association between SIRS and AKI in the subgroup of patients with non-paracetamol-induced ALF.

To our knowledge, this is the first study to examine the prevalence of SIRS in ALF using all four components of the systemic inflammatory response.²⁸ Previous groups have excluded the respiratory component because of the influence of mechanical ventilation.^{18–19,30} Using similar methodology to the earlier studies the prevalence of SIRS in our cohort was comparable (50%; Rolando *et al*, 57%; Vaquero *et al*, 34%; Schmidt *et al*, 55%). However, when all four components were applied, the prevalence of SIRS rose to 70%. The diagnosis of SIRS was based on parameters at the time of admission to our unit. Therefore, additional patients may have developed SIRS at a later stage.

The observation that SIRS may be present in patients with ALF even in the absence of infection confirms previous

Table 2 Clinical and biochemical characteristics on admission of patients with ALF who did/did not develop AKI

Variable	AKI (208)	Non-AKI (100)	p Value
Age (years)	41.0 (14.0)	36.9 (15.7)	0.020
Male:female	1:1.2	1:1.4	0.439
Alcohol excess (%)	88 (52)	30 (38)	0.043
Temperature ($^{\circ}\text{C}$)	36.4 (1.1)	36.8 (0.8)	0.001
Heart rate (bpm)	107 (92–120)	96 (80–110)	<0.001
WCC ($\times 10^9/\text{l}$)	12.8 (9.4–18.0)	11.0 (6.9–13.7)	0.001
Neutrophil count ($\times 10^9/\text{l}$)	11.2 (7.6–16.5)	8.8 (5.9–12.2)	<0.001
Lymphocyte count ($\times 10^9/\text{l}$)	0.7 (0.5–1.2)	1.0 (0.7–1.5)	0.002
Platelet count ($\times 10^9/\text{l}$)	115 (55–162)	158 (99–237)	<0.001
INR	4.9 (3.0–7.1)	3.7 (2.5–5.6)	0.001
Sodium (mmol/l)	134 (129–138)	136 (133–138)	0.007
Potassium (mmol/l)	4.1 (3.6–5.0)	3.6 (3.3–4.2)	<0.001
Urea (mmol/l)	7.7 (5.0–12.5)	4.1 (2.7–7.4)	<0.001
Creatinine ($\mu\text{mol/l}$)	221 (138–298)	92 (74–113)	<0.001
Bilirubin ($\mu\text{mol/l}$)	96 (68–139)	140 (88–433)	<0.001
ALT (U/l)	5856 (2105–10 000)	2655 (957–6775)	<0.001
Albumin (g/l)	32.9 (8.2)	32.9 (7.2)	0.946
H^+ (nmol/l)	41 (35–50)	33 (31–37)	<0.001
PaCO_2 (kPa)	4.0 (3.2–4.6)	4.2 (3.8–4.8)	0.033
HCO_3^- (mmol/l)	18.1 (13.7–22.8)	24.0 (20.9–26.9)	<0.001
Lactate (mmol/l)	4.2 (2.6–8.4)	2.7 (2.0–4.5)	0.007
Any encephalopathy (%)	125 (61)	68 (68)	0.253
SIRS (%)	136 (78)	43 (53)	<0.001

AKI, acute kidney injury; ALF, acute liver failure; ALT, alanine aminotransferase; INR, international normalised ratio; SIRS, systemic inflammatory response syndrome; WCC, white cell count.

Table 3 Factors predictive of AKI on univariate and multivariate analysis in all patients with ALF

Variable	Univariate OR (95% CI)	p Value	Multivariate OR (95% CI)	p Value
Age (years)	1.02 (1.00 to 1.04)	0.021	1.03 (1.00 to 1.06)	0.024
Paracetamol-induced ALF	3.80 (2.26 to 6.38)	<0.001	10.72 (4.24 to 27.09)	<0.001
KCH poor prognosis	3.08 (1.88 to 5.06)	<0.001	6.33 (2.65 to 15.13)	<0.001
Hypoglycaemia	4.44 (2.49 to 7.94)	<0.001		
Treatment for increased ICP	3.03 (1.60 to 5.73)	0.001		
Hypotension	11.23 (5.87 to 21.48)	<0.001	7.01 (3.06 to 16.07)	<0.001
SIRS	3.16 (1.80 to 5.57)	<0.001	2.42 (1.17 to 5.00)	0.017
Infection	2.07 (1.27 to 3.39)	0.004	1.93 (0.93 to 4.02)	0.077

Reference group (relative risk 1.00): non-paracetamol-induced ALF, did not achieve KCH prognostic criteria. No hypoglycaemia, no treatment for increased ICP, no hypotension, no SIRS, no infection.

AKI, acute kidney injury; ALF, acute liver failure; ICP, intracranial pressure; KCH, Kings College Hospital; SIRS, systemic inflammatory response syndrome.

findings.¹⁶⁻¹⁹ In fact, the prevalence of SIRS was similar in infected and non-infected groups. We acknowledge that occult infection may have influenced our results. However, our unit actively seeks infection with cultures performed routinely on admission, and repeated thereafter if clinically indicated. SIRS is the clinical sequelae of a massive inflammatory cascade that results from systemic cytokine release.³¹ SIRS occurs not only in infected patients but also in a variety of other conditions including trauma and acute pancreatitis.³¹ In ALF, non-infected patients similarly demonstrate high circulating levels of cytokines.³²⁻³⁵ The source of the systemic cytokines in this setting remains unclear, although release from the necrotic liver,

or secondary to endotoxaemia, or impaired hepatic cytokine metabolism are possible.³¹⁻³⁶⁻³⁷

The findings of our study suggest that the systemic inflammatory response plays a role in the pathogenesis of renal dysfunction in ALF. Consequently, the renal dysfunction of sepsis may be a more accurate parallel than the hepatorenal syndrome of cirrhosis. In sepsis, renal dysfunction probably occurs as a result of haemodynamic and non-haemodynamic factors.¹⁷ There is reduced glomerular filtration pressure, which reflects altered intrarenal and extrarenal vascular activity.¹⁷⁻²⁰ Furthermore, the systemic inflammatory response may contribute directly to renal tubular dysfunction by stimulating

Table 4 Univariate analysis of variables as predictors of AKI in patients with paracetamol-induced ALF and non-paracetamol-induced ALF

Variable	Paracetamol-induced ALF		Non-paracetamol-induced ALF	
	AKI (166)	Non-AKI (51)	AKI (42)	Non-AKI (49)
Peak creatinine ($\mu\text{mol/l}$)	341 (294–454)	109 (86–140)	329 (258–474)	99 (86–113)
Renal replacement therapy (%)	124 (75)	0	22 (52)	0
Age (years)	39.5 (13.1)	33.6 (14.2)**	47.4 (15.5)	40.3 (16.6)*
Male:female	1:1.0	1:1.2	1:2.0	1:1.7
Alcohol excess (%)	80 (57)	22 (54)		
Jaundice–encephalopathy time (days)			11 (2–25)	12 (2–23)
Temperature OA ($^{\circ}\text{C}$)	36.4 (1.1)	37.0 (0.9)***	36.3 (1.3)	36.6 (0.6)
Heart rate OA (bpm)	109 (92–120)	102 (86–120)	100 (90–120)	90 (80–100)**
WCC OA ($\times 10^9/\text{l}$)	13.0 (9.4–18.0)	11.5 (6.9–14.3)*	12.0 (9.4–18.0)	10.7 (7.0–12.8)*
Neutrophil count OA ($\times 10^9/\text{l}$)	11.5 (8.2–16.5)	10.5 (5.9–12.7)	9.6 (7.0–14.9)	7.7 (5.5–10.4)*
Platelet count OA ($\times 10^9/\text{l}$)	112 (47–157)	154 (92–220)**	136 (77–182)	164 (113–284)*
INR OA	5.3 (3.6–7.7)	4.6 (3.1–6.6)*	2.4 (1.9–4.3)	2.7 (2.0–4.2)
Peak INR	7.9 (5.0–10.7)	6.4 (4.3–9.3)	4.3 (2.2–5.1)	3.2 (2.3–5.1)
Bilirubin OA ($\mu\text{mol/l}$)	89 (62–116)	100 (74–124)	325 (110–531)	438 (241–524)
ALT OA (U/l)	6949 (3884–10 925)	6547 (4373–8790)	613 (171–2712)	1050 (365–2228)
Albumin OA (g/l)	34.3 (8.1)	36.5 (6.1)	28.1 (6.7)	29.2 (6.4)
H+ OA (nmol/l)	41 (35–50)	33 (30–38)***	41 (34–45)	34 (31–36)**
PaCO ₂ (kPa)	4.0 (3.2–4.6)	4.2 (3.7–4.8)	4.0 (3.4–5.1)	4.4 (3.8–4.7)
HCO ₃ ⁻ OA (mmol/l)	18.0 (13.0–22.4)	23.0 (20.0–26.9)***	20.3 (15.1–24.7)	24.6 (21.8–27.0)*
Lactate OA (mmol/l)	4.7 (2.8–9.0)	3.0 (2.1–5.3)	3.7 (2.3–7.0)	2.5 (1.7–4.0)
Hypotension (%)	102 (63)	5 (10)***	27 (64)	8 (17)***
Inotropes (%)	98 (60)	4 (8)***	18 (43)	5 (10)***
Hypoglycaemia (%)	89 (55)	11 (22)***	13 (31)	7 (15)
Treatment for increased ICP (%)	59 (36)	7 (14)**	10 (24)	7 (15)
Infection (%)	89 (55)	23 (46)	26 (63)	15 (31)**
SIRS (%)	110 (79)	28 (72)	26 (76)	15 (36)***
KCH poor prognosis (%)	105 (63)	0 (0)***	29 (69)	37 (76)
Spontaneous survival (%)	48 (37)	47 (92)***	8 (30)	10 (63)*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs no acute kidney injury group

AKI, acute kidney injury; ALF, acute liver failure; ALT, alanine aminotransferase; ICP, intracranial pressure; INR, international normalised ratio; KCH, Kings College Hospital; OA, on admission to hospital; SIRS, systemic inflammatory response syndrome; WCC, white cell count.

Hepatology

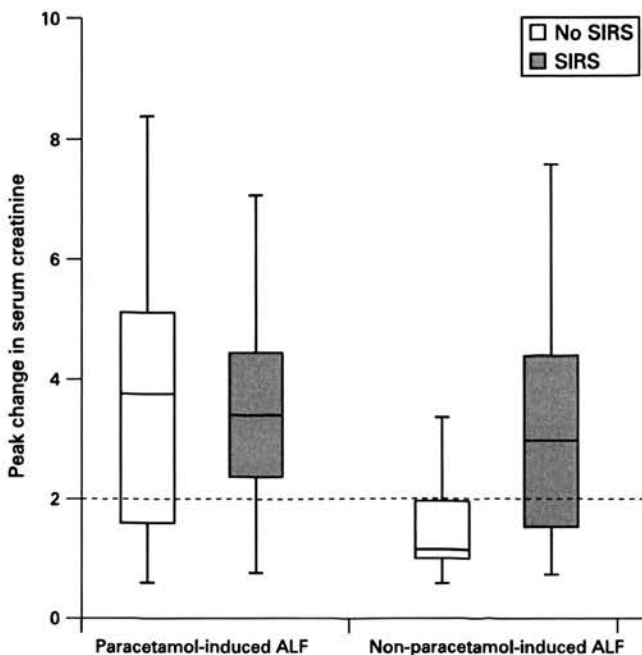


Figure 2 Boxplot of peak change in serum creatinine (peak serum creatinine/baseline serum creatinine) in patients with acute liver failure (ALF) subdivided based on aetiology, and presence or absence of the systemic inflammatory response syndrome (SIRS). A peak change in serum creatinine ≥ 2 , represented by the horizontal line, is the definition of acute kidney injury.

apoptotic death of tubular cells.^{17 38} Most research has focused on tumour necrosis factor α (TNF α). This cytokine appears to play a major role in the pathogenesis of the circulatory dysfunction and renal injury of sepsis.^{17 20 39} In ALF, TNF α and interleukin 6 (IL6) levels have been shown to correlate with the development of renal failure and circulatory failure, respectively.⁴⁰ Therefore, we propose that in ALF, as in sepsis, cytokines responsible for the systemic inflammatory response are central to the development of renal dysfunction.

The systemic inflammatory response has also been implicated in the pathogenesis of the circulatory and renal dysfunction of cirrhosis.^{41 42} These patients demonstrate endotoxaemia in the absence of infection and have increased systemic cytokine levels, which correlate with the severity of disease.^{43–48} Furthermore, prophylactic antibiotics reduce the incidence of hepatorenal syndrome and improve survival in patients with decompensated cirrhosis, independent of the prevention of infection.⁴⁹ Nevertheless, portal hypertension remains the primary event.^{4 50} Moreover, the frequency of SIRS in patients with cirrhosis, even when renal dysfunction is present (41%), is much less than we have demonstrated in ALF (AKI, 78%; non-AKI, 53%).⁴¹ Patients with subfulminant ALF are more likely to have clinically significant portal hypertension than those with fulminant ALF, and may develop ascites.⁹ Therefore, in subfulminant ALF, renal dysfunction may share similarities with hepatorenal syndrome of cirrhosis. However, in fulminant hepatic failure, the systemic inflammatory response may be the key mediator of renal dysfunction.

We were unable to demonstrate a relationship between the severity of ALF, as assessed by the KCH prognostic criteria, and SIRS. It is well recognised that there are significant inter-individual differences in the systemic inflammatory response to infection and other forms of injury, which may be genetically

predetermined.^{31 51} Therefore, it is likely that some individuals will be more at risk of the circulatory and renal complications of the systemic inflammatory response to ALF than others.

Paracetamol as the cause of ALF was an independent predictor of AKI, consistent with the clinical suspicion that paracetamol has a direct nephrotoxic effect. The mode of paracetamol-related nephrotoxicity remains undefined, although case reports of isolated renal failure following paracetamol overdose indicate that it is independent of hepatic injury.^{23 25} It is postulated that a locally produced toxic metabolite of paracetamol induces proximal tubular cell necrosis.^{23 24 52} Functional renal effects may also contribute as alterations in renal plasma flow and glomerular filtration rate have been demonstrated in the absence of structural and hepatic dysfunction.²⁴

Our study has some potential limitations. We chose to use Trey and Davidson's definition of ALF and include all patients with encephalopathy in the study. Grade I encephalopathy is difficult to diagnose, and previous similar studies have included patients with severe encephalopathy only.^{1 2 7} However, our cohort was comparable with the described populations with regards to severity of liver injury and outcome, and the diagnosis of encephalopathy was made by a small number of doctors, indicating relative consistency of opinion. Secondly, we did not have details of co-morbidities and, in particular, pre-existing renal disease or nephrotoxic medications that may have influenced our results. The patients studied were of a young age and it is therefore assumed that pre-existing renal function was normal. Our unit avoids nephrotoxic drugs when managing patients with ALF, although this does not preclude exposure prior to transfer.

The identification of modifiable risk factors for the development of AKI in ALF has important implications for patient management. As in sepsis, early and aggressive optimisation of haemodynamics with fluid therapy, central venous pressure monitoring and inotrope administration may significantly reduce the occurrence of renal dysfunction.^{20 53 54} Furthermore, manipulation of the systemic inflammatory response itself is likely to be advantageous. Prompt diagnosis and treatment of superimposed infection is essential.^{53 54} In addition, removal of cytokines and other inflammatory mediators by haemofiltration or albumin dialysis (MARS) may be beneficial.^{55–57} Finally, pentoxifylline, by downregulation of pro-inflammatory cytokines, is a possible future therapeutic option.^{58 59}

In conclusion, in this large single-centre retrospective study, we have examined the relationship between SIRS and AKI in ALF. We have shown that the SIRS is strongly associated with renal dysfunction and we hypothesise that the inflammatory cascade plays a key role in its pathogenesis. Given the reduced spontaneous survival of patients with AKI, we suggest that the early administration of therapies that target the systemic inflammatory response and limit the development of AKI may have a favourable effect on patient morbidity and mortality.

Competing interests: None.

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The systemic inflammatory response syndrome is predictive of renal dysfunction in patients with non-paracetamol-induced acute liver failure

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ORIGINAL ARTICLE

Is estimated glomerular filtration rate superior to serum creatinine in predicting mortality on the waiting list for liver transplantation?

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cirrhosis, estimated glomerular filtration rate, modification of diet in renal disease, survival.

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Conflicts of Interest

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Summary

Serum creatinine is an important prognostic indicator in patients on the liver transplant waiting-list, being a component of the Model for End Stage Liver Disease (MELD) score. However, creatinine is influenced by age, gender and race, and in this role may disadvantage some individuals. The Modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate (eGFR) takes into account these variables and may be a superior measure of renal function. Our aim was to examine whether the MDRD 4-variable, 5-variable and 6-variable eGFRs are superior to serum creatinine in predicting 3-month waiting-list mortality in patients with end-stage liver disease. This was a retrospective single-centre study of 427 adults listed for first liver transplantation. The median listing MDRD 4-variable, 5-variable and 6-variable eGFR was 69, 71 and 73 ml/min/1.73 m², respectively. The median listing serum creatinine was 89 µm. MDRD 4-variable ($P = 0.002$), 5-variable ($P < 0.001$) and 6-variable eGFR ($P < 0.001$), and serum creatinine ($P < 0.001$), were all predictors of mortality on the transplant waiting-list. Of the three MDRD equations, the 6-variable eGFR was the better prognostic indicator. The substitution of 6-variable eGFR for serum creatinine did not improve the prognostic accuracy of the MELD ($P = 0.825$) and UK score for Patients with End-Stage Liver Disease ($P = 0.781$) scores. In conclusion the MDRD eGFR is comparable, but not superior to serum creatinine, in predicting death within 3 months of listing for liver transplantation.

Cirrhosis is associated with a progressive functional renal impairment characterised by increased tubular sodium reabsorption, impaired free water clearance and prerenal azotemia [1]. This spectrum of renal dysfunction evolves in parallel with advancing disease and consequently the clinical manifestations of renal dysfunction, ascites, hyponatraemia and hepatorenal syndrome, are important prognostic markers [2–5]. Serum creatinine as a continuous variable is an independent predictor of mortality following the transjugular intrahepatic portosystemic shunt procedure and in those on the liver transplant waiting list [6,7]. It is a component of the Model For End-Stage Liver

Disease (MELD) score, which is used to prioritise graft allocation.

However, serum creatinine is not solely influenced by glomerular filtration and is not an accurate estimator of renal function [8]. Creatinine production is proportional to muscle mass, and is greater in men than in women, in younger than older individuals and in black people than in white people, despite similar glomerular filtration rate [9]. In addition, in cirrhosis reduced creatine production by the liver, muscle wasting and increased renal tubular secretion of creatinine may result in a falsely low serum creatinine level [10,11].

The effect of gender, age and race on serum creatinine is of particular concern in the MELD era of organ allocation. United Network for Organ Sharing (UNOS) data has demonstrated that women listed for liver transplantation are less likely to survive to transplantation than men, supporting a systematic bias of the scoring system [12–14]. Similarly, an inherent discrimination against older patients could explain the independent association of increasing age with waiting-list mortality [15]. It follows that a scoring system with an alternative measure of renal function may be preferable to MELD.

The gold standard measure of glomerular filtration rate, inulin clearance, has recently been shown to be superior to serum creatinine in predicting liver transplant waiting-list mortality [16]. Unfortunately, inulin clearance is time consuming, impractical and costly and is not a useful test if repeated measures are required [10,11]. Calculated glomerular filtration rate is a possible alternative and has been evaluated as an absolute measure of renal function, although not as a prognostic marker, in this setting [17].

The most accurate calculated glomerular filtration rate for cirrhotic patients is provided by the Modification of Diet in Renal Disease study (MDRD) equations, which are creatinine-based estimates modified for age, gender and race [17–19]. The MDRD 4-variable calculated glomerular filtration rate is readily available, at minimal cost, with routine reporting advocated in several countries, and is an attractive measure of renal function [20,21]. The MDRD 5-variable and 6-variable calculated glomerular filtration rates, in addition, adjust for blood urea nitrogen, and blood urea nitrogen and serum albumin, respectively, and could be superior prognostic indicators.

The aim of our study was to examine whether the MDRD calculated glomerular filtration rate is superior to serum creatinine in predicting prognosis on the liver transplant waiting list. In a subgroup of patients measured creatinine clearance (CrCl) was also available and was examined as a prognostic indicator.

Methods

This was a single-centre retrospective study of consecutive adults listed for first liver transplantation between November 1992 and June 2007. Patients listed for acute liver failure, hepatocellular carcinoma, or joint liver/kidney transplantation, or who had documented intrinsic renal disease were not assessed. Those removed from or still active on the waiting list were also not included.

The following variables at time of liver transplant assessment were recorded: gender, age, race, aetiology of liver disease, presence of ascites or hepatic encephalopathy and laboratory data (serum sodium, creatinine, bilirubin,

albumin and international normalised ratio). Estimated glomerular filtration rate was calculated from the relevant parameters using the MDRD 4-variable [eGFR (MDRD4) = $186 \times \text{creatinine (mg/dl)}^{-1.154} \times \text{age (years)}^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$], MDRD 5-variable [eGFR (MDRD5) = $270 \times \text{creatinine (mg/dl)}^{-1.007} \times \text{age (years)}^{-0.180} \times \text{blood urea nitrogen (mg/dl)}^{-0.169} \times (0.755 \text{ if female}) \times (1.178 \text{ if black})$] and MDRD 6-variable [eGFR (MDRD6) = $170 \times \text{creatinine (mg/dl)}^{-0.999} \times \text{age (years)}^{-0.176} \times \text{blood urea nitrogen (mg/dl)}^{-0.170} \times \text{albumin (g/dl)}^{+0.318} \times (0.762 \text{ if female}) \times (1.180 \text{ if black})$] equations [18,19]. The MELD score was determined as previously described [22]. The UK Score for Patients with End-Stage Liver Disease (UKELD), a recently devised scoring system that incorporates serum sodium in addition to the MELD variables, was also calculated [23].

In a subgroup of patients transplanted between May 2000 and June 2007 CrCl was available. This was determined from a 24-h urinary collection performed routinely during the in-patient assessment period. Failure to obtain a CrCl was, in most cases, secondary to poor patient compliance.

Statistical analyses

Normally distributed continuous variables and nonparametric continuous variables were compared using the Student's *t*-test and Mann–Whitney test, respectively. Chi-square analysis was used for the comparison of categorical variables. Survival modelling was performed using Cox proportional hazards regression. Data was censored at the time of liver transplantation and to lessen the influence of extreme values all continuous laboratory variables were transformed into their natural logarithms. To allow the comparison of MELD or UKELD with a similar model with logeGFR or logeCrCl substituted for logecreatinine the regression coefficients of MELD or UKELD were initially adjusted for our patient population. Regression coefficients were then recalculated in the presence of logeGFR instead of logecreatinine. Receiver-operating characteristic (ROC) curves were generated to assess the accuracy of models in predicting 3-month waiting-list mortality. Concordance statistics were compared using the method described by Hanley and McNeil [24]. All patients censored prior to the specified time point were excluded from these analyses. A value of $P < 0.05$ was considered statistically significant at all times. Data were analysed using the SPSS 15 package (SPSS Inc., Chicago, IL, USA).

Values are expressed as mean and standard deviation (SD), and median and inter-quartile range (IQR) as appropriate.

Results

Patient characteristics

The mean age of the patients ($n = 427$) at time of listing for liver transplantation was 55.3 (SD 11.6) years and the male to female ratio was 1:1. The main indications for transplantation were primary biliary cirrhosis (119 patients, 27.9%), alcoholic liver disease (103 patients, 24.1%), sclerosing cholangitis (62 patients, 14.5%), hepatitis C cirrhosis (37 patients, 8.9%) cryptogenic cirrhosis (36 patients, 8.4%) and autoimmune hepatitis (33 patients, 7.7%). The median listing MELD score was 16 (IQR 13–20) and the median listing UKELD score was 56 (IQR 54–60).

Sixty patients (14.1%) died prior to liver transplantation. The median time from listing to death was 50 (IQR 26–101) days. For patients who were transplanted the median waiting-time was 68 (IQR 27–142) days. Two hundred and twelve patients (49.6%) were transplanted and 44 patients (10.3%) died within 3 months of listing.

The median listing serum creatinine was 89 (IQR 77–107) μM , the median listing serum sodium was 136 (IQR 132–139) mM , and 60.6% of patients had ascites. The median eGFR (MDRD4), eGFR (MDRD5) and eGFR (MDRD6) was 69 (IQR 57–83) ml/min/1.73 m^2 , 71 (IQR 56–86) ml/min/1.73 m^2 , and 73 (IQR 57–89) ml/min/1.73 m^2 , respectively.

Comparison of MDRD equations as predictors of waiting list mortality

Loge creatinine (OR 14.12; 95% CI 3.76–53.13, $P < 0.001$), logeGFR (MDRD4) (OR 0.18; 95% CI 0.06–0.53, $P = 0.002$), logeGFR (MDRD5) (OR 0.16; 95% CI 0.06–0.44, $P < 0.001$), and logeGFR (MDRD6) (OR 0.14; 95% CI 0.05–0.39, $P < 0.001$) demonstrated an association with 3-month waiting-list mortality.

Receiver-operating characteristic curves for loge creatinine, logeGFR (MDRD4), logeGFR (MDRD5) and logeGFR (MDRD6) as predictors of 3-month waiting list mortality are shown in Fig. 1. When all eGFR equations were compared logeGFR (MDRD6) had the greatest concordance statistic [logeGFR (MDRD4) 0.648; 0.548–0.749; logeGFR (MDRD5) 0.683; 0.587–0.780; logeGFR (MDRD6) 0.695; 0.601–0.789, loge creatinine 0.696; 0.598–0.793, c-statistic and 95% confidence interval]. LogeGFR (MDRD6) statistically outperformed logeGFR (MDRD4) ($P = 0.054$), and was comparable to logeGFR (MDRD5) ($P = 0.614$) and loge creatinine ($P = 0.981$). Following on from this, all further analyses comparing eGFR with serum creatinine were performed using the eGFR MDRD 6-variable equation.

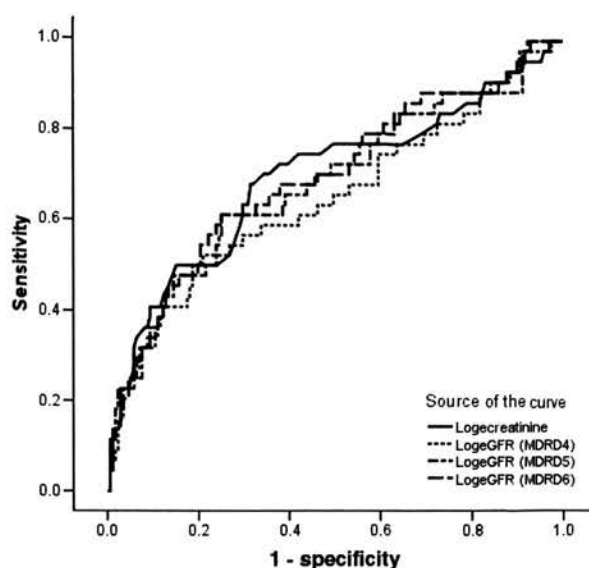


Figure 1 Receiver-operating characteristic curves of log serum creatinine (loge creatinine), log eGFR calculated using the Modification of Diet in Renal Disease (MDRD) 4-variable equation [logeGFR (MDRD4)], log eGFR calculated using the MDRD 5-variable equation [logeGFR (MDRD5)] and log eGFR calculated using the MDRD 6-variable equation [logeGFR (MDRD6)] for predicting 3-month liver transplant waiting list mortality.

Does substitution of eGFR (MDRD6) for serum creatinine improve the prognostic accuracy of MELD and UKELD?

ROC analysis was used to determine whether the substitution of logeGFR (MDRD6) for loge creatinine improved the accuracy of the existing prognostic models, MELD and UKELD (Table 1). The regression coefficients for

Table 1. AUC for receiver-operating characteristic curves for prediction of 3-month liver transplant waiting-list mortality in all patients.

Model	c-statistic	95% CI
MELD (adj)	0.841	0.773–0.909
MELD (eGFR)	0.846	0.777–0.915
UKELD (adj)	0.859	0.790–0.928
UKELD (eGFR)	0.864	0.795–0.933

eGFR, estimated glomerular filtration rate; MELD, Model for End-Stage Liver Disease; MELD (adj), MELD score with regression coefficients adjusted for our model; UKELD, UK score for Patients with End-Stage Liver Disease; UKELD (adj), UKELD score with regression coefficients adjusted for our model; MELD (eGFR), MELD score with logeGFR substituted for loge creatinine; UKELD (eGFR), UKELD score with logeGFR substituted for loge creatinine; c-statistic, concordance statistic.

each model were initially adjusted for our study population [MELD (adj)/UKELD (adj)], and thereafter recalculated in the presence of logeGFR (MDRD6) instead of logecreatinine [MELD (eGFR)/UKELD (eGFR)].

The LogeGFR (MDRD6) substituted for logecreatinine did not change the concordance statistic for MELD as a predictor of 3-month waiting-list mortality [MELD (adj) versus MELD (eGFR), $P = 0.825$]. Furthermore, logeGFR (MDRD6) substituted for logecreatinine did not alter the concordance statistic for UKELD as a predictor of death by 3 months [UKELD (adj) versus UKELD (eGFR), $P = 0.781$].

In view of the concern that the MELD and UKELD scoring systems are systemically biased and may discriminate against female and older patients the concordance statistics of individual patient groups were also determined (Table 2). There was no statistically significant difference in the concordance statistics of the MELD score or UKELD score between genders (MELD, $P = 0.718$; UKELD, $P = 0.645$) and age groups (MELD, $P = 0.099$; UKELD, $P = 0.216$). LogeGFR (MDRD6) substituted for logecreatinine did not change the concordance statistic for MELD or UKELD as predictors of 3-month waiting-list mortality in female, male, older or younger patients (P values not shown).

Does substitution of CrCl for serum creatinine improve the prognostic accuracy of MELD and UKELD?

Measured creatinine clearance was available in 139 of the 256 patients (54.3%) listed for liver transplantation between May 2000 and June 2007. The CrCl patients were comparable to patients who did not have a recorded CrCl (Table 3). In this cohort of 139, 31 patients (22.3%) died prior to transplantation. The median time from listing to death was 49 (IQR 19–88) days. The median waiting-time

to transplantation was 85 (IQR 35–179) days. Fifty-five patients (39.6%) were transplanted and 25 patients (18.0%) died within 3 months of listing.

The median listing serum creatinine, serum sodium, eGFR (MDRD6) and CrCl was 91 (IQR 79–110) μM , 136 (IQR 131–139) mM , 75 (60–87) ml/min/1.73 m^2 , and 72 (51–95) ml/min , respectively. CrCl demonstrated a greater correlation with eGFR (MDRD6) (0.615, $P < 0.001$) than with serum creatinine (-0.452 , $P < 0.001$).

Logecreatinine (OR 7.77, 95% CI 1.33–45.51, $P = 0.023$) and logeCrCl (OR 0.22, 95% CI 0.07–0.67,

Table 3. Comparison of listing variables in patients listed for liver transplantation between May 2000 and June 2007 who did and did not have measured creatinine clearance available.

Variable	No CrCl (n = 117)	CrCl (n = 139)	P-value
Age (years)	54.0 (12.3)	55.3 (11.4)	0.374
Male gender	62 (53.0)	85 (61.2)	0.188
Noncholestatic disease	71 (60.7)	83 (59.7)	0.874
INR	1.4 (1.2–1.6)	1.3 (1.1–1.6)	0.553
Bilirubin (μM)	76 (42–139)	84 (46–156)	0.526
Albumin (g/l)	28.9 (5.4)	29.0 (5.4)	0.876
Encephalopathy	36 (43.9)	35 (42.7)	0.875
Ascites	60 (60.6)	70 (60.3)	0.969
Sodium (mM)	135 (132–138)	136 (131–139)	0.591
Creatinine (μM)	91 (78–106)	91 (79–106)	0.795
eGFR (MDRD6)	73 (58–90)	76 (60–87)	0.997
MELD	17 (14–20)	16 (14–21)	0.771
UKELD	57 (54–61)	57 (54–61)	0.936

Values expressed as mean (standard deviation), median (inter-quartile range) and number (percentage) where appropriate.

Units for eGFR (MDRD6) = ml/min/1.73 m^2 .

CrCl, measured creatinine clearance; INR, international normalised ratio; MDRD, Modification of Diet in Renal Disease; MELD, Model for End-Stage Liver Disease; eGFR (MDRD6), estimated glomerular filtration rate derived from 6-variable MDRD equation.

Table 2. AUC for receiver-operating characteristic curves for prediction of 3-month liver transplant waiting-list mortality in different patient groups.

Model	Females		Males		Older		Younger	
	c-statistic	95% CI	c-statistic	95% CI	c-statistic	95% CI	c-statistic	95% CI
MELD	0.807	0.677–0.938	0.775	0.660–0.890	0.734	0.612–0.857	0.872	0.764–0.981
MELD (adj)	0.847	0.740–0.955	0.820	0.723–0.917	0.810	0.703–0.916	0.882	0.797–0.967
MELD (eGFR)	0.848	0.733–0.962	0.843	0.752–0.933	0.791	0.677–0.906	0.880	0.788–0.971
UKELD	0.794	0.664–0.924	0.833	0.729–0.936	0.771	0.659–0.884	0.876	0.755–0.988
UKELD (adj)	0.826	0.712–0.940	0.874	0.784–0.963	0.833	0.731–0.936	0.891	0.794–0.989
UKELD (eGFR)	0.828	0.715–0.941	0.879	0.790–0.967	0.825	0.719–0.931	0.891	0.788–0.993

Older defined as age ≥ 60 years, younger defined as age < 60 years.

eGFR, estimated glomerular filtration rate; MELD, standard Model for End-Stage Liver Disease score; UKELD, standard UK score for Patients with End-Stage Liver Disease score; MELD (adj), MELD score with regression coefficients adjusted for our model; UKELD (adj), UKELD score with regression coefficients adjusted for our model; MELD (eGFR), MELD score with logeGFR substituted for logecreatinine; UKELD (eGFR), UKELD score with logeGFR substituted for logecreatinine; c-statistic, concordance statistic.

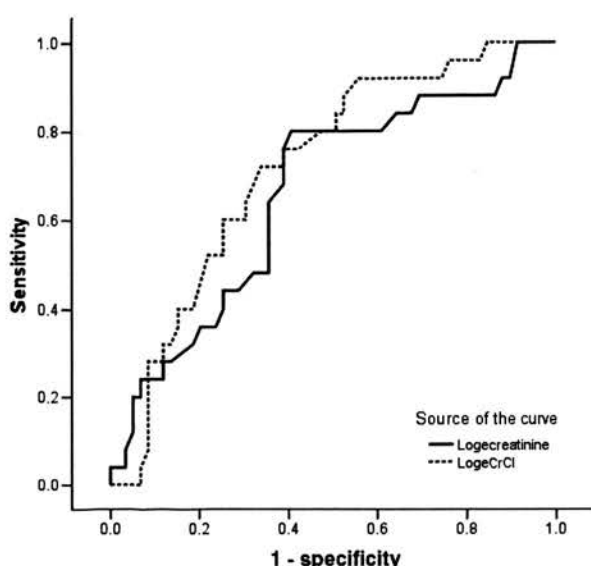


Figure 2 Receiver-operating characteristic curves of log serum creatinine (logcreatinine) and log creatinine clearance (logeCrCl) for predicting 3-month liver transplant waiting list mortality.

$P = 0.008$) were associated with 3-month waiting-list mortality. The ROC curves for logcreatinine and logeCrCl are shown in Fig. 2. Logcreatinine and logeCrCl had similar concordance statistics for the prediction of death by 3 months (logcreatinine 0.660; 0.532–0.788; logeCrCl 0.718; 0.604–0.831, c-statistic and 95% confidence interval, $P = 0.353$).

As before, ROC analysis was used to determine whether the substitution of logeCrCl for logcreatinine improved the accuracy of the existing prognostic models, MELD and UKELD (Table 4). LogeCrCl substituted for logcreatinine did not change the concordance statistic for MELD [MELD (adj) versus MELD (CrCl), $P = 0.249$] or

Table 4. AUC for receiver-operating characteristic curves for prediction of 3-month liver transplant waiting-list mortality.

Model	c-statistic	95% CI
MELD (adj)	0.809	0.708–0.910
MELD (CrCl)	0.845	0.765–0.926
UKELD (adj)	0.849	0.756–0.942
UKELD (CrCl)	0.881	0.808–0.954

CrCl, measured creatinine clearance; MELD, Model for End-Stage Liver Disease; MELD (adj), MELD score with regression coefficients adjusted for our model; UKELD, UK score for Patients with End-Stage Liver Disease; UKELD (adj), UKELD score with regression coefficients adjusted for our model; MELD (CrCl), MELD score with logeCrCl substituted for logcreatinine; UKELD (CrCl), UKELD score with logeCrCl substituted for logcreatinine; c-statistic, concordance statistic.

UKELD [UKELD (adj) versus UKELD (CrCl), $P = 0.198$] as a predictor of 3-month waiting-list mortality.

Discussion

Our study has examined for the first time eGFR, calculated using the MDRD equations, in the prediction of mortality on the liver transplant waiting list. We have demonstrated that decreasing eGFR, as a continuous variable, was associated with an increased risk of death within 3 months of listing. This reiterates the well recognised spectrum of renal dysfunction that occurs in the setting of cirrhosis and reflects the underlying circulatory derangement of advanced disease. Of the three MDRD equations, the eGFR derived from the 6-variable equation was the better prognostic indicator. On univariate analysis, eGFR (MDRD6) was comparable, but not superior, to listing serum creatinine for prediction of 3-month waiting-list mortality. When substituted for serum creatinine eGFR (MDRD6) did not improve the prognostic accuracy of the existing MELD and UKELD models.

Although a negative study, the finding that eGFR (MDRD6) is not superior to serum creatinine in the prediction of waiting-list mortality is an important observation. Several studies have previously highlighted the prognostic inadequacies of serum creatinine in patients with end-stage liver disease [12,13]. Concerns have been raised that scoring systems for graft allocation that incorporate serum creatinine may disadvantage some individuals. In searching for alternative measures of renal function the next step is to use creatinine-based estimates of glomerular filtration rate that adjust for patient factors potentially conferring systemic bias. The MDRD eGFR is well validated in the nonliver setting, is calculated from readily available variables including age, gender and race, and has been shown to be the most accurate eGFR in cirrhotic patients [9,17–19]. Our negative results support the need for further research to identify more precise noncreatinine-based measures of renal function in these patients.

An explanation for the failure of eGFR (MDRD6) to improve the MELD and UKELD scoring systems is that the equation does not take into account disease-related factors such as nutritional status. Consequently, eGFR (MDRD6) is not an accurate measure of absolute renal function with one-third of patients demonstrating an MDRD estimate outwith 30% of the measured glomerular filtration rate [17]. The Cockcroft–Gault eGFR adjusts for body weight and, although a less precise estimator of glomerular filtration rate in this population, its ability to predict survival remains unknown [17,25]. Notably, the difficulty in obtaining an accurate dry weight in patients with significant ascites and peripheral oedema makes the Cockcroft–Gault eGFR a less attractive option [10,11].

Other possible weaknesses of eGFR for predicting mortality on the liver transplant waiting list are as follows: similar to ascites and serum sodium concentration, eGFR may be influenced by diuretic use and could theoretically be subject to manipulation [2]. Furthermore, a reduced eGFR may reflect intrinsic renal disease, which may not confer the same prognostic significance. All patients with evidence of renal impairment should have renal pathology excluded with urinalysis and renal imaging [4]. Creatinine assays are not currently standardised and there is significant variability in serum creatinine levels using different methods [26]. Therefore, the prognostic significance of eGFR may not be echoed in all centres.

The association of CrCl with mortality in patients listed for liver transplantation was a further novel finding of this study. Decreasing CrCl, as a continuous variable, was associated with an increased risk of death within 3 months of listing. Mirroring the findings of eGFR (MDRD6) CrCl was a comparable, but not superior prognostic indicator to serum creatinine. When substituted for serum creatinine CrCl increased the accuracy of MELD and UKELD by 3.6% and 3.2%, respectively, although statistical significance was not achieved. The negative result may reflect a relatively small patient subgroup, but probably reflects the inaccuracy of CrCl as a measure of absolute renal function [27].

Despite the large population assessed in this single-centre study, we recognise some potential limitations. Firstly, because of the retrospective nature we cannot ensure that all patients with intrinsic renal disease were excluded from the analysis. In our unit, patients assessed for liver transplantation routinely undergo urine testing and renal ultrasonography, and those with significant renal impairment are considered for renal biopsy. As a result, most patients with intrinsic renal disease should have been identified. Secondly, biochemical values were based on a single measurement and may not have been a true representation of the steady state in all. However, during the 5-day liver transplant assessment our patients are relatively stable and less likely to be subject to diuretic-induced or sepsis-related acute renal impairment. Thirdly, the patients included in the study were listed over a 15-year period during which advances have been made in the management of chronic liver disease, such as the widespread use of terlipressin and albumin for hepatorenal syndrome. Therefore, there may be a small time effect that could not be factored into the statistical analysis. Finally, the indications for transplantation in this cohort differ somewhat from the typical transplant centre with a greater proportion of patients listed for primary biliary cirrhosis and less for viral hepatitis. The MELD score has been shown to have comparable 3-month mortality risk prediction in a diverse range of liver diseases, both chole-

static and noncholestatic [7]. Consequently, we do not believe that the somewhat atypical spread of aetiologies should have influenced our findings.

Clinically applicable, precise measures of renal function are not currently available in cirrhotic patients. Serum creatinine remains the most widely used parameter and despite its limitations has some clinical relevance. A change in serum creatinine may indicate haemodynamic decompensation or intrinsic renal disease, and serum creatinine is an important prognostic indicator [6,7]. In this study we have demonstrated that listing eGFR (MDRD6) was comparable, but not superior, to listing serum creatinine for prediction of 3-month waiting-list mortality, and when substituted for serum creatinine eGFR (MDRD6) did not improve the prognostic accuracy of the existing MELD and UKELD models. Our findings support the need for further research to identify more precise noncreatinine-based measures of renal function.

Authorship

JAL: research design, data collection, data analysis and paper writing. SMMK: data collection. JWF and PCH: research design and paper writing.

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Chronic Kidney Disease After Liver Transplantation for Acute Liver Failure Is Not Associated With Perioperative Renal Dysfunction

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Renal dysfunction of acute liver failure (ALF) may have distinct pathophysiological mechanisms to hepatorenal syndrome of cirrhosis. Yet, the impact of perioperative renal function on posttransplant renal outcomes in ALF patients specifically has not been established. The aims of this study were (1) to describe the incidence and risk factors for chronic renal dysfunction following liver transplantation for ALF and (2) to compare renal outcomes with age–sex-matched patients transplanted for chronic liver disease. This was a single-center study of 101 patients transplanted for ALF. Fifty-three-and-a-half percent had pretransplant acute kidney injury and 64.9% required perioperative renal replacement therapy. After transplantation the 5-year cumulative incidence of chronic kidney disease (eGFR <60 mL/min/1.73 m²) was 41.5%. There was no association between perioperative acute kidney injury ($p = 0.288$) or renal replacement therapy ($p = 0.134$) and chronic kidney disease. Instead, the independent predictors of chronic kidney disease were older age ($p = 0.019$), female gender ($p = 0.049$), hypertension ($p = 0.031$), cyclosporine ($p = 0.027$) and nonacetaminophen-induced ALF ($p = 0.039$). Despite marked differences in the perioperative clinical condition and survival of patients transplanted for ALF and chronic liver disease, renal outcomes were the same. In conclusion, in patients transplanted for ALF the severity of perioperative renal injury does not predict posttransplant chronic renal dysfunction.

Key words: Acute kidney injury, acute liver failure, chronic kidney disease, transplant

Abbreviations: ALF, acute liver failure; SIRS, systemic inflammatory response syndrome; INR, international normalized ratio; eGFR, estimated glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; SD, standard deviation; IQR, interquartile range; HR, hazard ratio; CI, confidence interval; MELD, Model for End-

Stage Liver Disease; CNI, calcineurin inhibitor; RRT, renal replacement therapy; CLD, chronic liver disease.

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Renal dysfunction is a common complication of acute liver failure (ALF) with two-thirds of patients manifesting acute kidney injury, and almost half requiring renal replacement therapy (1). Many have postulated that the pathogenesis is similar to the hepatorenal syndrome of cirrhosis (2,3). However, a growing body of evidence supports a systemic inflammatory response to ALF, and the systemic inflammatory response syndrome (SIRS) is an independent predictor of acute kidney injury in ALF patients (1,4,5). It follows that the renal dysfunction of sepsis may be a more accurate parallel than the hepatorenal syndrome (1). Additional factors that may contribute to renal dysfunction in ALF but are less likely in stable cirrhotic patients include hypovolemia, nephrotoxic drugs particularly acetaminophen, infection and disseminated intravascular coagulation (6–8).

Despite the contrasting perioperative clinical condition of patients transplanted for ALF and chronic liver disease (CLD), post-liver transplant renal outcomes have not been examined specifically in this group. Pretransplant glomerular filtration rate, pretransplant renal failure requiring renal replacement therapy and acute renal injury are consistent predictors of chronic renal dysfunction after elective liver transplantation (9,10). Given the greater baseline circulatory and neuro-humoral derangement of ALF it seems possible that the acute hemodynamic effects of the calcineurin inhibitors administered immediately following transplantation are exaggerated (11–14). On the other hand, the differing pathophysiological mechanisms could offer relative reno-protection and a reduced risk of chronic kidney disease.

The clarification of the impact of liver transplantation for ALF on posttransplant renal function has important implications for patient management. Chronic renal dysfunction is a major cause of patient morbidity and mortality and the minimization of renal injury has emerged as a priority for transplant physicians (9,15–17). Simultaneous liver–kidney

transplantation is not an option in patients transplanted for ALF because of the medical urgency, but the identification of prognostic variables could help to determine those who may benefit from tailored renal sparing immunosuppressive regimens (18).

The aims of this study were first to describe the incidence and risk factors for chronic renal dysfunction following liver transplantation for ALF and second to compare renal outcome with an age–sex-matched group of patients transplanted for CLD.

Methods

This was a retrospective single-center study of consecutive patients who underwent super-urgent liver transplantation for ALF (UK Transplant Super Urgent Scheme Category 1–7) between December 1992 and July 2007 (19). Eight patients had inadequate documentation available and were excluded from the analysis. A further 1 patient was lost to follow-up. Therefore, the study cohort comprised 101 patients. The causes of ALF were acetaminophen (46 patients, 45.5%), seronegative hepatitis (27 patients, 26.7%), idiosyncratic drug reaction (11 patients, 10.9%), autoimmune hepatitis (7 patients, 6.9%), hepatitis B (4 patients, 4.0%), Budd-Chiari (3 patients, 3.0%), Wilson's disease (2 patients, 2.0%) and hepatitis A (1 patient, 1.0%).

ALF was defined as severe liver injury with hepatic encephalopathy in which the onset of encephalopathy was within 8 weeks for the first symptoms of illness, and in the absence of preexisting liver disease (20).

Data were collected on the following preoperative variables at the time of listing: age, gender, race, liver disease etiology, additional comorbidity, smoking status, international normalized ratio (INR), serum bilirubin, albumin, serum creatinine, serum sodium (hyponatremia; sodium <135 mmol/L) and presence of ascites (on ultrasound). SIRS was defined as ≥ 2 of temperature <36°C or >38°C, heart rate >90 beats per minute, white cell count <4 × 10⁹/L or >12 × 10⁹/L and PaCO₂ < 4.3 kPa at the time of admission (21). Documented perioperative variables were peak preoperative serum creatinine, preoperative renal replacement therapy, postoperative renal replacement therapy, inotropes (noradrenaline/adrenaline), bacterial sepsis and fungal sepsis. Immunosuppression was noted and calcineurin inhibitor trough levels at 1 week, 1 month and 12 months (a comparable 12-month value for the linear regression analysis was obtained for all patients regardless of calcineurin inhibitor by expressing the trough as relative to the median value). Renal function was recorded at 1 month, 6 months, 12 months and 2, 3, 4 and 5 years following transplantation. Patients still receiving renal replacement therapy at 1 month were given an arbitrary serum creatinine of 350 µmol/L and an estimated glomerular filtration rate (eGFR) of 15 mL/min/1.73 m².

A patient was considered to have significant renal dysfunction preoperatively if they fulfilled the RIFLE criteria for acute kidney injury: peak serum creatinine ≥ 2 times the baseline level (22). The baseline serum creatinine was unavailable for most patients and was estimated as previously described (1,22). Following transplantation the main measure of renal function was eGFR, determined using the Modification of Diet in Renal Disease (MDRD) Study 4-variable equation ($\text{eGFR} = 186 \times \text{creatinine (mg/dL)}^{-1.154} \times \text{age (years)}^{-0.203} \times 1.210 \text{ (if black)} \times 0.742 \text{ (if female)}$) (23). Chronic kidney disease was defined as eGFR < 60 mL/min/1.73 m² on at least 2 occasions from 6 months posttransplant onwards: stage 3, stage 4 and stage 5

chronic kidney disease were defined as eGFR 30–59 mL/min/1.73 m², 15–29 mL/min/1.73 m² and <15 mL/min/1.73 m² or on dialysis, respectively (24).

To examine whether the renal dysfunction of ALF has a different renal prognosis after transplantation to the renal dysfunction of CLD a control group of patients transplanted for CLD was identified. These patients were age-matched (to within 5 years) and sex-matched to the original cohort. The relatively young age of the patients transplanted for ALF meant that only 71 patients could be appropriately matched. The causes of CLD were primary biliary cirrhosis (18 patients, 25.4%), alcohol (10 patients, 14.1%), chronic active hepatitis (9 patients, 12.7%), sclerosing cholangitis (9 patients, 12.7%), cryptogenic cirrhosis (9 patients, 12.7%), hepatitis C (5 patients, 7.0%) and other (11 patients, 15.5%). Three patients (4.2%) were transplanted for hepatocellular carcinoma. None of the control patients had intrinsic renal disease prior to transplantation and no patient underwent combined liver–kidney transplantation.

Immunosuppression was similar for patients transplanted for ALF and for CLD, and consisted of a calcineurin inhibitor, azathioprine and prednisolone in most cases. Midway through the specified time period the unit policy for calcineurin inhibitor changed from cyclosporine to tacrolimus. Prednisolone was usually discontinued by 3 to 6 months posttransplant unless otherwise indicated. Deviation from the protocol occurred only in the setting of adverse event or graft rejection. Acute rejection was usually managed with 1 g of methyl-prednisolone intravenously for 3 days followed by reintroduction of oral steroids with or without increased dose of, or switch to, alternative calcineurin inhibitor. Chronic rejection was managed with the latter and in a small number of patients azathioprine was changed to mycophenolate. Interleukin (IL)-2 receptor antagonist induction therapy was not administered to any of the patients.

Statistical analyses

Cumulative incidence of chronic kidney disease was estimated using the Kaplan–Meier method. Survival was estimated using Kaplan–Meier plots with log-rank test for differences, and age-adjusted survival was determined using Cox proportional hazards analyses. Normally distributed continuous variables and nonparametric continuous variables were compared using the Student's t-test and Mann–Whitney test, respectively. Chi-squared analysis or Fisher's exact test were used for comparison of categorical data. A multivariate linear regression analysis was performed to explore the relationship between perioperative renal dysfunction and long-term renal function following transplantation. Clinically relevant factors were included simultaneously with 12-month eGFR as the dependent variable. Cox proportional hazards analysis was then used to identify variables predictive of chronic kidney disease by 5-years posttransplant. Three multivariate models were constructed with all clinically relevant factors entered simultaneously. Variables entered into Model 1 were age, gender, pretransplant diagnosed hypertension, category of ALF (acetaminophen-induced vs. nonacetaminophen-induced), SIRS, calcineurin inhibitor at time of hospital discharge and pretransplant acute kidney injury. In Models 2 and 3 acute kidney injury was replaced by the other measures of perioperative renal dysfunction, peak preoperative change in serum creatinine and immediate posttransplant renal replacement therapy, respectively. All three measures of perioperative renal dysfunction were not included in the same model because of collinearity. None of the multivariate models was adjusted for the presence of pretransplant diabetes mellitus secondary to small patient numbers. $p < 0.05$ was considered statistically significant unless otherwise stated. Data were analyzed using the SPSS 15 package (SPSS Inc., Chicago, IL, USA).

All values are expressed as mean and standard deviation (SD), and median and interquartile range (IQR) as appropriate.

Table 1: Clinical characteristics of acute liver failure patients at time of listing for liver transplantation (n = 101)

Clinical characteristics	
Baseline demographics	
Age (years)	36.3 (14.1)
Male : Female	1:1.5
Caucasian	99 (98)
Comorbidity	
Diagnosed hypertension	6 (5.9)
Type I diabetes mellitus	2 (2.0)
Type II diabetes mellitus	1 (1.0)
Dyslipidaemia	1 (1.0)
Cause of ALF	
Acetaminophen	46 (45.5)
Seronegative hepatitis	27 (26.7)
Idiosyncratic drug reaction	11 (10.9)
Autoimmune hepatitis	7 (6.9)
Hepatitis B	4 (4.0)
Budd-Chiari	3 (3.0)
Wilson's disease	2 (2.0)
Hepatitis A	2 (1.0)
Clinical characteristics at listing	
Bilirubin ($\mu\text{mol/L}$)	193 (80–463)
INR	8.3 (3.2–11.7)
Albumin (g/L)	30.8 (10.2)
Creatinine ($\mu\text{mol/L}$)	160 (94–298)
Sodium (mmol/L)	135 (5)
MELD score	43 (35–52)
Ascites	24 (23.8)
SIRS	59 (70.2)
Grade III/IV encephalopathy	57 (59.4)
Inotropes	36 (37.9)

Values expressed as mean (standard deviation), median (interquartile range) and number (percent) where appropriate.

ALF = acute liver failure; INR = international normalised ratio; MELD = Model for End-Stage Liver Disease; SIRS = systemic inflammatory response syndrome.

Results

Patient characteristics

The median jaundice to encephalopathy time for patients with nonacetaminophen-induced ALF was 14 (IQR 11–31) days. In patients with acetaminophen-induced ALF the median time from overdose to listing for liver transplantation was 69 (IQR 54–72) h. Patient characteristics at the time of listing are outlined in Table 1. The median time from listing to transplantation was 1 (IQR 1–2) day. The estimated 1-month, 12-month and 5-year posttransplant patient survival was 83%, 75% and 68%, respectively.

Perioperative renal function

Actual baseline renal function was available in 21 patients: the median baseline serum creatinine was 75 (IQR 60–93) $\mu\text{mol/L}$ and the mean baseline eGFR was 106 (SD 45) mL/min/1.73 m².

During the immediate preoperative period the median peak serum creatinine of the entire cohort was 203 (IQR 102–362) $\mu\text{mol/L}$. Fifty-four patients (53.5%) fulfilled the criteria for acute kidney injury, of whom 72.2% underwent renal replacement therapy. A further five patients were commenced on hemofiltration in the absence of a creatinine rise. Following transplantation 64.9% (n = 63) received renal replacement therapy. By 1-month posttransplant the median serum creatinine was 97 (IQR 83–136) $\mu\text{mol/L}$ and the mean eGFR was 67 (SD 40) mL/min/1.73 m². Four of the surviving patients (4.8%) were still on renal replacement therapy at this time point.

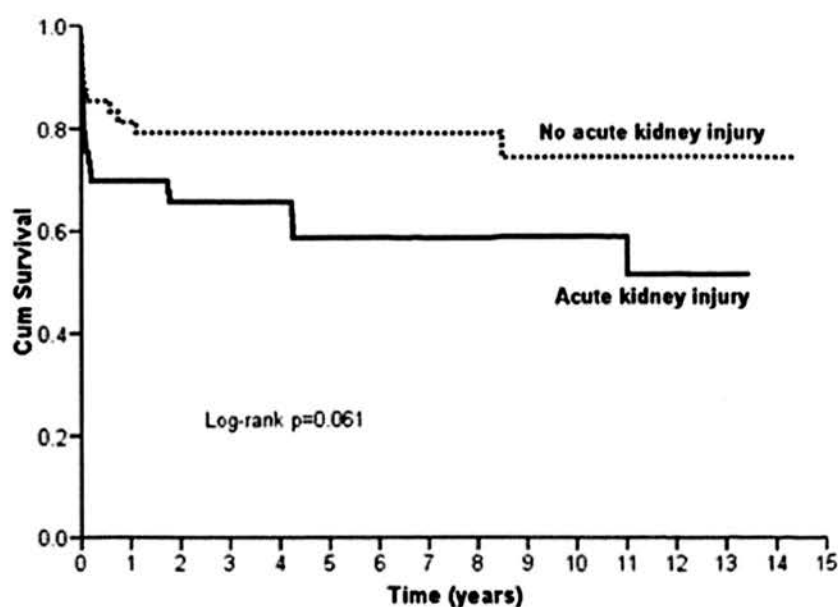
When patients with and without acetaminophen-induced ALF were compared the former were more likely to demonstrate perioperative renal dysfunction. Acetaminophen-induced ALF patients had a greater median peak preoperative serum creatinine (acetaminophen-induced-ALF, 332 (217–415) $\mu\text{mol/L}$, n = 47; nonacetaminophen-induced ALF, 108 (86–187) $\mu\text{mol/L}$, median (IQR), n = 54; p < 0.001), a greater frequency of acute kidney injury (acetaminophen-induced ALF, 83%; nonacetaminophen-induced ALF, 28%; p < 0.001) and a greater frequency of pre-(acetaminophen-induced ALF, 74%; nonacetaminophen-induced ALF, 15%; p < 0.001) and postoperative renal replacement therapy (acetaminophen-induced ALF, 95%; nonacetaminophen-induced ALF, 38%; p < 0.001). At 1-month posttransplant mean eGFR was similar for the two groups (acetaminophen-induced ALF, 57 (30) mL/min/1.73 m², n = 36; nonacetaminophen-induced ALF, 74 (44) mL/min/1.73 m²; mean (SD), n = 48; p = 0.053).

Postoperative renal function

In most patients renal function demonstrated maximal recovery by 6- to 12-months following transplantation. The mean 12-month eGFR was 70 (SD 21) mL/min/1.73 m², and 21.1% (n = 16) of patients had stage 3–5 chronic kidney disease by this time point. In those patients with follow-up up to 5 years after transplantation the mean eGFR remained stable at 70 (SD 20) mL/min/1.73 m², and the prevalence of stage 3–5 chronic kidney disease was 29.5% (n = 13). Twelve-month eGFR demonstrated a close correlation with 5-year eGFR (r = 0.809, p < 0.001). The cumulative incidence of stage 3–5, and stage 4–5 chronic kidney disease by 5 years was 41.5% and 2.6%, respectively.

Relationship between perioperative renal dysfunction and posttransplant mortality

ALF patients who fulfilled the criteria for acute kidney injury prior to transplantation had greater mortality post transplant (log-rank p = 0.061; age-adjusted HR 2.09; 95% CI 1.01–4.34, p = 0.048; Figure 1). Similarly, patients who required postoperative renal replacement therapy demonstrated an increased risk of death (age-adjusted HR 6.22; 95% CI 2.01–19.26, p = 0.002).



Number of patients at risk:

No acute kidney injury

48 36 26 21 16 10 6 3

Acute kidney injury

53 31 23 19 12 10 4 0

Figure 1: Kaplan-Meier plot of the probability of survival following liver transplantation for acute liver failure subdivided based on the presence or absence of preoperative acute kidney injury.

Relationship between perioperative renal dysfunction and posttransplant renal function

To explore the relationship between perioperative renal dysfunction and long-term renal function following transplantation for ALF a multiple linear regression analysis was performed, therefore, allowing adjustment for other relevant clinical factors such as age, gender and immunosuppressive therapy. Given the close correlation between 12-month eGFR and 5-year eGFR, 12-month eGFR was used as the dependent variable (Table 2). The analysis revealed no significant association between pretransplant acute kidney injury and 12-month posttransplant eGFR ($p = 0.098$). Instead, increasing age ($p = 0.012$), female gender ($p = 0.005$), preoperative SIRS ($p = 0.041$) and cyclosporine as primary immunosuppression ($p = 0.021$) were associated with worse renal function. Patients with acetaminophen-induced ALF had a higher 12-month eGFR compared with patients with nonacetaminophen-induced ALF ($p = 0.027$).

Predictors of chronic kidney disease following transplantation

Recognizing the significant morbidity and mortality of chronic kidney disease, as well as concerns regarding the influence of early deaths after transplantation on the 12-

month data, Cox regression was then performed to identify perioperative variables predictive of posttransplant chronic kidney disease. Variables associated with the development of chronic kidney disease following transplantation on univariate analysis are outlined in Table 3. A subsequent multivariate regression analysis including all clinically relevant variables simultaneously (Model 1 Table 3) identified older age (overall $p = 0.019$), female gender ($p = 0.049$), pretransplant diagnosed hypertension ($p = 0.031$) and cyclosporine immunosuppressive therapy ($p = 0.027$) to be predictors of chronic kidney disease after transplantation. Patients transplanted for acetaminophen-induced ALF were at lower risk of chronic kidney disease than patients transplanted for nonacetaminophen-induced ALF ($p = 0.039$).

Pretransplant AKI both on univariate analysis ($p = 0.796$), and after adjusting for confounding factors ($p = 0.288$), was not predictive of posttransplant chronic kidney disease. Similarly, no relationship was demonstrated between peak preoperative change in serum creatinine (univariate analysis, $p = 0.838$; multivariate analysis, $p = 0.457$, Model 2 Table 3), or renal replacement therapy during the immediate posttransplant period (univariate analysis, $p = 0.420$; multivariate analysis, $p = 0.134$, Model 3 Table 3) and chronic renal dysfunction.

Table 2: Multivariate linear regression analysis of variables associated with eGFR 12-months following liver transplantation for acute liver failure

Variable	B	(95% CI)	β	p-Value
Age (years)	-0.516	-0.916, -0.116	-0.318	0.012
Female gender	-14.500	-24.358, -4.643	-0.332	0.005
Past medical history:	-20.354	-59.134, 18.426	-0.123	0.297
hypertension				
Acetaminophen-induced ALF	16.176	1.889, 30.463	0.371	0.027
Preoperative SIRS	-12.780	-24.991, -0.570	-0.270	0.041
Preoperative acute kidney injury	-10.204	-22.369, 1.960	-0.235	0.098
CNI: cyclosporine	-12.465	-22.947, -1.983	-0.263	0.021
12-month CNI trough	0.583	-7.662, 8.832	0.016	0.887

Reference group (relative risk 1.00): male gender, past medical history: no hypertension, nonacetaminophen-induced ALF, no preoperative SIRS, no preoperative acute kidney injury, CNI: tacrolimus (at time of 12-month eGFR). Bold indicates statistical significance.

ALF = acute liver failure; B = unstandardized regression coefficient; β = standardized regression coefficient; CNI = calcineurin inhibitor; SIRS = systemic inflammatory response syndrome.

Comparison of posttransplant renal function in patients transplanted for ALF and age-sex matched patients transplanted for CLD

To determine whether ALF per se is associated with renal function following transplantation age-sex-matched patients transplanted for CLD were introduced into the statistical analysis. Pre- and perioperative characteristics of patients with ALF and with CLD are compared in Table 4. The median waiting-list time was 1 (IQR 1–2) day for ALF patients and 52 (IQR 20–136) days for CLD patients ($p < 0.001$). Median listing serum creatinine was higher ($p < 0.001$), mean listing eGFR lower ($p < 0.001$) and the frequency of ascites less ($p < 0.001$) in the ALF group. Furthermore, the ALF patients were more likely to receive pre- ($p < 0.001$) and postoperative renal replacement therapy ($p < 0.001$). The estimated 1-month, 12-month and 5-year survival was 78%, 69% and 62%, respectively, for ALF patients and 96%, 90% and 79% for patients with CLD (Figure 2, log-rank $p = 0.029$).

By 1 month following transplantation the median serum creatinine (ALF, 92 [83–127] $\mu\text{mol/L}$; CLD, 88 [79–101] $\mu\text{mol/L}$, median [IQR]; $p = 0.103$) and the mean eGFR (ALF, 69 [42] mL/min/1.73 m^2 ; CLD, 74 [27] mL/min/1.73 m^2 , mean [SD]; $p = 0.451$) were similar in ALF and CLD patients. Figure 3 illustrates the mean pre- and posttransplantation eGFR in patients with ALF and CLD surviving to 12 months. The accompanying table (Table 5) documents relevant pre- and posttransplant clinical variables

of these surviving patients. Despite significantly lower listing eGFR in the ALF group renal function was similar at all time points in the postoperative period. The cumulative incidence of stage 3–5 (ALF, 48.7%; CLD, 49.6%; $p = 0.930$) and stage 4–5 chronic kidney disease (ALF, 4.1%; CLD, 8.7%; $p = 0.615$) by 5 years was also no different between ALF and CLD groups.

Discussion

In this large single-center study we have described for the first time the risk factors for chronic renal dysfunction following emergency liver transplantation for ALF. Importantly, we have shown that perioperative kidney injury does not appear to have negative consequences for long-term renal function in this population. Contrary to observations in CLD patients pretransplant acute kidney injury and renal replacement therapy were not associated with chronic kidney disease. Only failure to recover renal function, as evidenced by eGFR at 1-month posttransplant, was a predictive factor. Despite marked differences in the perioperative clinical condition of patients transplanted for ALF and CLD long-term renal outcome following transplantation was the same.

The rate of chronic kidney disease after transplantation for ALF was similar to that reported by Aberg et al., the single other publication of renal function in this setting (25). Although half of our patients fulfilled the criteria for acute kidney injury pretransplant, and more than 60% required renal replacement therapy during the immediate postoperative period, only 21% had an eGFR less than $60\text{ mL/min/1.73 m}^2$ 12 months thereafter. This dramatic renal recovery echoes clinical observations in spontaneous survivors of ALF. By 5-years posttransplant the cumulative incidence of chronic kidney disease was 42%.

The identical posttransplant renal function of ALF and CLD patients was unexpected when considering our current understanding of the underlying mechanisms (11–14,26). Based on the traditional hypothesis of hepatorenal syndrome one might predict that severe perioperative renal vasoconstriction would exacerbate calcineurin-inhibitor mediated kidney dysfunction (11–14,26). Cyclosporine and tacrolimus cause an initially hemodynamic dose-dependent renal impairment that is feasibly exaggerated in patients with greater baseline circulatory and neuro-humoral derangement (11–14). Our results support an alternative patho-physiological process underlying the renal injury that occurs in ALF.

We have previously demonstrated that SIRS predicts the development of acute kidney injury in patients with nonacetaminophen-induced ALF, a relationship that appears to be independent of the severity of liver injury (1). Consequently, we have postulated that the renal dysfunction of sepsis may be a more accurate parallel than

Table 3: Univariate and multivariate cox regression analysis of variables as predictors of chronic kidney disease (eGFR <60 mL/min/1.73m²) by 5-years following liver transplantation for acute liver failure

	Univariate		Multivariate Model 1		Multivariate Model 2		Multivariate Model 3	
	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
Age								
<30 years	1.00		1.00		1.00		1.00	
30–44 years	3.59 (1.10–11.67)	0.034	4.14 (1.18–14.50)	0.026	4.18 (1.19–14.65)	0.026	4.32 (1.27–14.62)	0.019
≥45 years	11.35 (3.63–35.51)	< 0.001	5.94 (1.62–21.74)	0.007	6.11 (1.67–22.36)	0.006	8.15 (2.08–31.96)	0.003
Female gender	2.23 (0.95–5.25)	0.067	2.94 (1.00–8.64)	0.049	2.86 (0.96–8.58)	0.060	2.92 (1.02–8.34)	0.045
Diagnosed hypertension	2.39 (0.57–10.11)	0.236	14.16 (1.28–156.2)	0.031	12.17 (1.12–131.89)	0.040	12.95 (1.20–140.22)	0.035
ALF	1.00		1.00		1.00		1.00	
Non POD	0.42 (0.18–0.95)	0.037	0.23 (0.06–0.93)	0.039	0.26 (0.07–1.01)	0.052	0.22 (0.06–0.81)	0.022
POD								
Preoperative:								
SIRS	1.41 (0.56–3.52)	0.467	2.11 (0.63–7.12)	0.229	2.09 (0.63–7.01)	0.231	1.93 (0.57–6.52)	0.288
Acute kidney injury	0.91 (0.43–1.91)	0.796	1.70 (0.64–4.51)	0.288				
Peak Δ creatinine	0.97 (0.75–1.26)	0.838			1.14 (0.81–1.61)	0.457		
Postoperative:								
CNI on discharge								
Tacrolimus	1.00		1.00		1.00		1.00	
Cyclosporine	2.00 (0.95–4.19)	0.067	2.67 (1.12–6.37)	0.027	2.67 (1.12–6.36)	0.026	2.20 (0.88–5.48)	0.091
RRT	0.74 (0.35–1.55)	0.420					2.39 (0.77–7.44)	0.134
1 month eGFR (mL/min/1.73 m ²)								
≥90	1.00							
60–89	11.63 (1.50–90.24)	0.019						
30–59	12.02 (1.52–95.17)	0.018						
<30	18.95 (2.33–154.4)	0.006						

Reference group (relative risk 1.00): male gender, past medical history: no hypertension, no SIRS, no acute kidney injury, no RRT. Bold indicates statistical significance. ALF = acute liver failure; CNI = calcineurin inhibitor; eGFR = estimated glomerular filtration rate; peak Δ creatinine = peak change in serum creatinine (peak serum creatinine / baseline serum creatinine); RRT = renal replacement therapy during immediate postoperative period; SIRS = systemic inflammatory response syndrome.

Table 4: Pre- and perioperative clinical characteristics of patients transplanted for acute liver failure and age–sex-matched patients transplanted for chronic liver disease

Characteristic	ALF patients (n = 71)	CLD patients (n = 71)	p-Value
Age (years)	42.2 (12.4)	42.5 (12.1)	0.918
Male:Female	1:1.3	1:1.3	1.000
Time on waiting list (days)	1 (1–2)	52 (20–136)	<0.001
Comorbidity			
Diagnosed hypertension	6 (8.5)	2 (2.8)	0.137
Type I diabetes mellitus	0 (0)	(0)	1.000
Type II diabetes mellitus	1 (1.4)	5 (7.0)	0.104
Dyslipidemia	1 (1.4)	4 (5.6)	0.183
Hepatitis C	1 (1.4)	6 (8.5)	0.058
Active smoker	37 (56.9)	20 (29.4)	0.001
At listing			
INR	5.8 (2.8–11.1)	1.2 (1.1–1.5)	<0.001
Bilirubin (μmol/L)	227 (81–486)	67 (35–172)	<0.001
Albumin (g/L)	30.8 (11.4)	30.0 (5.6)	0.582
MELD score	41 (33–52)	16 (12–19)	<0.001
Creatinine (μmol/L)	148 (94–298)	81 (71–97)	<0.001
eGFR (mL/min/1.73 m ²)	49 (42)	83 (30)	<0.001
Hyponatremia	27 (38.0)	19 (26.8)	0.151
Ascites	18 (25.4)	41 (57.7)	<0.001
Perioperative			
Peak creatinine (μmol/L)	200 (99–384)		
Pre RRT	30 (42.3)	0 (0)	<0.001
Post RRT	41 (61.2)	12 (16.9)	<0.001
Fungal sepsis	5 (7.5)	3 (4.2)	0.327
Bacterial sepsis	35 (52.2)	25 (35.2)	0.044
Super-urgent retransplant	4 (5.6)	4 (5.6)	0.641
Early acute cellular rejection	17 (25.4)	28 (39.4)	0.078
CNI on discharge: cyclosporin	16 (28.1)	27 (39.1)	0.192

Values expressed as mean (standard deviation), median (interquartile range) and number (percent) where appropriate.

ALF = acute liver failure; CLD = chronic liver disease; CNI = calcineurin inhibitor; eGFR = estimated glomerular filtration rate; INR = international normalized ratio; MELD = Model for End-Stage Liver Disease; RRT = renal replacement therapy.

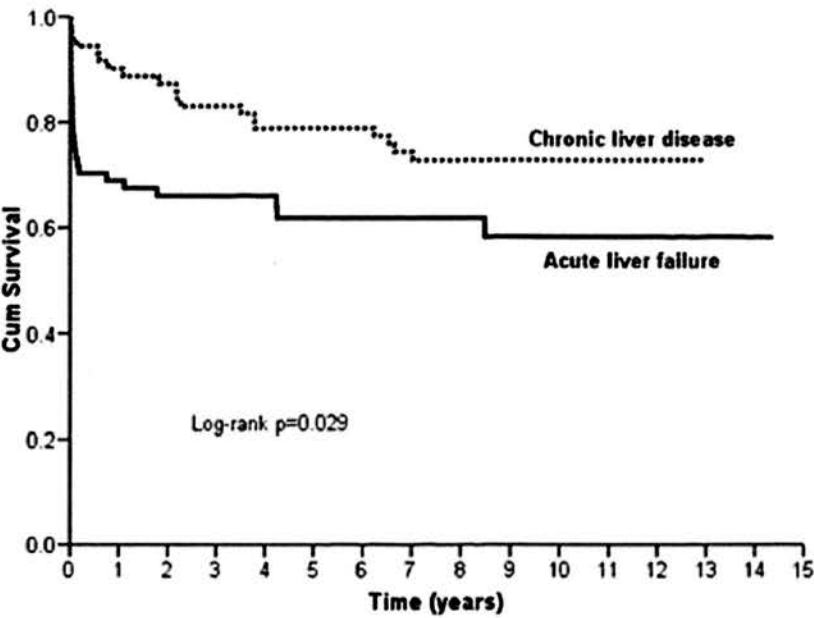
Table 5: Relevant pre- and posttransplant clinical characteristics of all patients transplanted for acute liver failure and patients transplanted for chronic liver disease surviving to 12 months after transplant

Characteristic	ALF patients (n = 49)	CLD patients (no = 62)	p-Value
Age at listing (years)	41.8 (12.4)	42.5 (12.4)	0.772
Male:Female	1:1	1:1.3	0.569
Comorbidity at 12-month posttransplant			
Diagnosed hypertension	14 (28.6)	17 (27.4)	0.893
Type II diabetes mellitus	7 (14.3)	7 (11.3)	0.637
Insulin-dependent diabetes mellitus	1 (2.0)	6 (9.7)	0.103
CNI on discharge: cyclosporine	16 (32.7)	25 (40.3)	0.406
1 week CNI trough:			
Cyclosporine	181 (57)	145 (52)	0.157*
Tacrolimus	8.5 (3.3)	10.1 (3.1)	0.195*
1 month CNI trough:			
Cyclosporine	151 (39)	162 (48)	0.492*
Tacrolimus	10.8 (4.6)	8.1 (3.2)	0.006*
12 month CNI trough:			
Cyclosporine	133 (40)	160 (40)	0.073*
Tacrolimus	8.0 (3.3)	7.9 (2.8)	0.974*

Values expressed as mean (standard deviation) and number (percent) where appropriate.

*p-value <0.017 considered significant.

ALF = acute liver failure; CLD = chronic liver disease; CNI = calcineurin inhibitor.



Number of patients at risk:

Chronic liver disease

71 62 56 56 33 13 1 0

Acute liver failure

71 43 32 23 26 11 4 1

Figure 2: Kaplan-Meier plot of the probability of survival following liver transplantation for acute liver failure and chronic liver disease.

the hepatorenal syndrome of cirrhosis (1). In fulminant hepatic failure the systemic inflammatory response may be the key mediator of renal impairment. Patients with subfulminant ALF are more likely to have clinically signif-

icant portal hypertension, and may develop ascites (27). Therefore, this group may share some of the hemodynamic and neuro-humoral features of hepatorenal syndrome (1). In sepsis, kidney injury may occur in the setting of

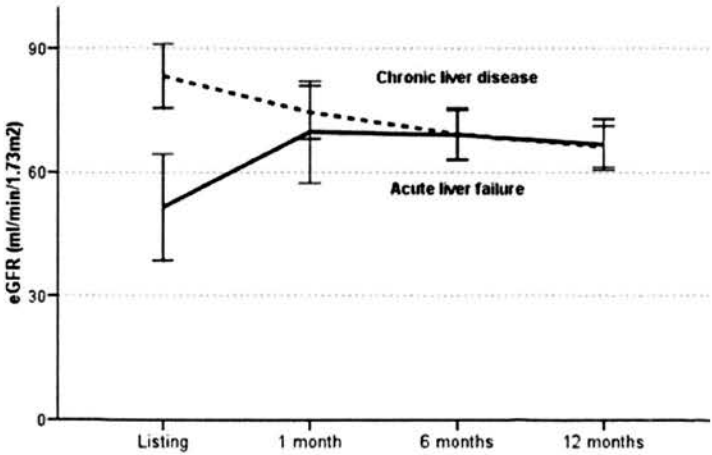


Figure 3: Mean estimated glomerular filtration rate (eGFR) and 95% confidence intervals at the time of listing for liver transplantation and at 1, 6 and 12 months following transplantation in all patients surviving to 12 months subdivided into acute liver failure and chronic liver disease groups. p -value <0.013 considered significant. The median time from listing to transplantation for acute liver failure patients was 1 (IQR 1–2) day and for chronic liver disease patients was 52 (20–136) days.

eGFR (ml/min/1.73m²) compared at time points

Chronic liver disease (n:62)	83.3	74.5	69.2	66.2
Acute liver failure (n:49)	51.1	69.6	69.0	66.7
p value	<0.001	0.468	0.965	0.897

preserved or even increased renal perfusion, which is in contrast to the intense renal vasoconstriction of hepatorenal syndrome (26,28). We propose that relative renal hyperemia may help to minimize the renal hemodynamic response to calcineurin inhibitors and explain the comparable long-term posttransplant renal function demonstrated by ALF patients (14).

Alternatively, the failure of perioperative renal dysfunction to impact on long-term posttransplant renal outcomes may reflect the duration of renal impairment. In patients transplanted for CLD renal dysfunction duration appears to be a key determinant of chronic renal impairment. Campbell et al. demonstrated that renal dysfunction duration of greater than 3.6 weeks pretransplant was an appropriate cut-off to identify patients at risk of renal insufficiency 12-months thereafter (29). In our cohort the renal injury, although more severe, was on the contrary short-lived.

In the nontransplant population acute kidney injury is a risk factor for chronic renal dysfunction. For example, in patients who undergo major vascular surgery the occurrence of perioperative acute kidney injury is associated with an increased risk of chronic kidney disease (30). Furthermore, patients requiring dialysis for acute kidney injury who are dialysis-independent at the time of hospital discharge are three times more likely to develop end-stage renal failure (31). Animal studies have confirmed that acute kidney injury can cause permanent structural kidney damage with progressive tubulo-interstitial fibrosis and long-term implications for renal function (32). Our failure to show a relationship between perioperative renal dysfunction and posttransplant chronic kidney disease is not in accordance with these observations. Acute kidney injury is an independent predictor of mortality in patients with ALF, and following transplantation for ALF (1). Yet, our findings suggest that beyond hospital discharge acute renal impairment, if short-lived, does not impact particularly on chronic renal function.

Acetaminophen as the cause of ALF was associated with a higher absolute eGFR at 12-months following transplantation and a reduced risk of chronic kidney disease. Acetaminophen is an independent predictor of acute kidney injury in patients with ALF and there are case reports of renal failure following acetaminophen overdose in the absence of significant hepatic injury (1,33,34). Animal models support a direct nephrotoxic effect although the mechanism remains unclear (33). It has been hypothesized that a locally produced metabolite induces proximal tubular cell necrosis while functional renal effects may also contribute (33,35,36). Our findings support the reversibility of acetaminophen-induced nephrotoxicity (37,38).

The study has some potential limitations that should be mentioned. First, baseline renal function was only avail-

able in a small number of ALF patients and it is possible that a proportion could have had undiagnosed intrinsic renal disease. The patients studied were of a relatively young age and it is assumed that premorbid renal function was normal. Second, nephrotoxic medications could have influenced the severity of perioperative renal dysfunction. Our unit avoids nephrotoxic drugs, yet this does not preclude exposure prior to transfer. Third, although our study consists of one of the largest single center cohorts of patients transplanted for ALF it remains possible that the relatively small numbers may have influenced our results.

With regards the CLD group, only 70% of the ALF patients could be matched because of the young age range. Furthermore, the pretransplant eGFR was only available at the time of listing and not immediately prior to transplantation. Pretransplant kidney function may, therefore, have been over represented in the CLD patients if there was a significant deterioration on the list. Nevertheless, no CLD patient required preoperative renal replacement therapy or reassessment for combined liver-kidney transplantation and, given the relatively short median waiting-list time of 52 days, it seems unlikely that this data would have influenced the results. The lack of pretransplant renal impairment in the control arm may also raise some concerns about its generalizability for a standard population of liver transplant recipients. This largely reflects the younger age of the patients. However, those with intrinsic renal disease or who received a simultaneous liver-kidney transplant were also deliberately excluded; we wished to examine whether the physiological differences between ALF and CLD would influence renal outcomes. Of course, it is well recognized that eGFR is not an accurate measure of renal function in patients listed for elective liver transplantation, tending to overestimate when the true GFR is reduced (39). Sixty percent of the CLD patients had ascites and one-third had hyponatraemia, indicating a high prevalence of portal hypertensive-related renal impairment (40). Finally, it is difficult to ensure retrospectively that ALF and CLD patients received similar immunosuppressive regimes. However, during the period studied our unit had a single protocol that was rarely deviated from with calcineurin inhibitor administration within 24 h of transplantation. The similar posttransplant calcineurin inhibitor trough levels support this claim.

The findings of our study have important implications for patient management. Patients who undergo liver transplantation for ALF should not be considered a high-risk group for developing chronic kidney disease even when perioperative acute renal impairment is severe. Consequently, we do not support the routine use of interleukin-2 receptor antagonists and delayed introduction of the calcineurin inhibitor in this setting (18). Renal sparing immunosuppression such as mycophenolate and reduced dose tacrolimus could be considered in select patients, for example older females transplanted for nonacetaminophen-induced ALF (41).

In conclusion, in this large single-center study of patients transplanted for ALF we have shown that the severity of perioperative renal dysfunction was not predictive of post-transplant chronic kidney disease. Despite greater perioperative physiological derangement in ALF patients when compared with an age–sex-matched cohort transplanted for CLD renal function following transplantation was the same.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Modifiable patient factors are associated with the late decline in renal function following liver transplantation

Leithead JA, Ferguson JW, Hayes PC. Modifiable patient factors are associated with the late decline in renal function following liver transplantation.

Abstract: Strategies to delay or avoid the long-term decline in renal function and progression to chronic kidney disease (CKD) in liver transplant recipients remain unclear. Our aim was to examine the change in estimated GFR (eGFR) from six months after liver transplantation, and to identify modifiable factors associated with a faster rate of decline. This was a single-center retrospective study of 97 patients who underwent elective liver transplantation and survived ≥ 5 yr. eGFR was estimated using the MDRD6-variable equation, and the annualized change in eGFR was determined using simple linear regression. The baseline eGFR was 75 mL/min/1.73 m². Thereafter, eGFR declined at a mean rate of 1.08 mL/min/1.73 m² per year. 49% had a decline in renal function greater than the rate expected with aging. Decline in eGFR was an independent predictor of CKD by five yr post-transplant ($p = 0.001$). Multivariate modeling found a higher baseline eGFR ($p < 0.001$), female gender ($p = 0.006$), hypertension ($p = 0.019$), and dyslipidemia ($p = 0.034$) to be associated with a faster rate of decline in renal function. In conclusion, liver transplant recipients have a clinically relevant decline in eGFR from six months post-transplant. Prospective studies are required to examine the effects of aggressive blood pressure and lipid control on the development of CKD in this setting.

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Key words: chronic kidney disease – decline – glomerular filtration rate – liver transplantation – progression

Abbreviations: Δ GFR, mean annualized change in eGFR from six months to five yr post-transplant; BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; CNI, calcineurin inhibitor; eGFR, estimated GFR; GFR, glomerular filtration rate; IQR, inter-quartile range; MDRD, modification of diet in renal disease study; MELD, model for end-stage liver disease; OR, odds ratio; RRT, renal replacement therapy; SD, standard deviation

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Chronic renal dysfunction is an important complication of liver transplantation, associated with significant morbidity and mortality (1–3). The five-yr cumulative incidence of severe chronic kidney disease (CKD) has been reported to be as high as 18%, and liver transplant recipients who develop CKD have a five times increased risk of death (1, 4). The prevention of chronic renal dysfunction following liver transplantation has emerged as a priority for transplant physicians.

Early postoperative renal function is an indicator of renal outcome in this setting. Most patients demonstrate a steep decline in glomerular filtration rate (GFR) during the first postoperative weeks with relative stabilization thereafter, and

six months and 12 months post-transplant GFR are consistent predictors of chronic renal dysfunction (1, 5–9). Consequently, the perioperative period has become a focus for interventions to lessen the decline in GFR. Cyclosporine and tacrolimus are considered to play a critical role in shaping renal function after transplantation (6, 10–12). Several randomized controlled trials examining perioperative minimization of calcineurin inhibitor exposure have been published (13–15). Yet, the results are mixed failing to demonstrate any convincing long-term benefit in unselected patients.

The change in renal function beyond the initial postoperative period has received less attention. The steady-state long-term rate of change in GFR

allows individuals at risk of progression to CKD to be identified (16, 17). Furthermore, by examining the relationship between rate of change and modifiable risk factors, potential therapeutic interventions can be suggested (16, 17). In renal transplant recipients, patients who receive tacrolimus or non-calcineurin inhibitor-based immunosuppression have a slower rate of decline in renal function compared with patients prescribed cyclosporine (18). In non-transplant CKD, hypertension, poor glycemic control, smoking, and possibly dyslipidemia have been linked with a faster decline in GFR (17). Renal biopsies of liver transplant patients with chronic renal failure support a multifactorial origin (19). However, the impact of such characteristics on the long-term rate of change in GFR and the development of renal dysfunction following liver transplantation remains unclear.

The aim of this study was to examine the long-term change in GFR from six months following liver transplantation, and to identify modifiable factors associated with a faster rate of decline.

Methods

This was a retrospective single-center case-note study of patients who underwent elective first liver transplantation between January 1st 1996 and December 31st 2000. The specified time period allowed the analysis of patients transplanted in an experienced center with a follow-up time post-transplant of >5 yr. This was an era when perioperative renal sparing immunosuppression, and aggressive alteration of immunosuppression in response to moderate post-transplant renal dysfunction, was infrequently practiced in our unit. Furthermore, cardiovascular risk factors were not as closely monitored. Therefore, variability of management between patients was less. To allow the analysis of change in renal function in a relatively homogeneous cohort of patients, the records of the following groups were not reviewed: those listed for acute liver failure ($n = 39$) or joint liver/kidney transplantation ($n = 7$), those who did not survive for five or more years post-transplant ($n = 33$), and those who changed calcineurin inhibitor therapy during the follow-up period ($n = 9$). Eighteen patients had incomplete data available and were also excluded from the analysis. The study group comprised a total of 97 patients.

Immunosuppression consisted of a calcineurin inhibitor, azathioprine, and prednisolone in most patients. Midway through the specified time period, the unit policy for calcineurin inhibitor changed from cyclosporine to tacrolimus. Prednisolone was usually discontinued by 3–6 months

post-transplant unless otherwise indicated. Deviation from the protocol occurred only in the setting of adverse event or graft rejection. Acute rejection was usually managed with 1 g of methylprednisolone intravenously for three d followed by reintroduction of oral steroids with or without increased dose of, or switch to, alternative calcineurin inhibitor. Chronic rejection was managed with the latter, and in seven patients, azathioprine was changed to mycophenolate.

Data were collected on the following at the time of listing for liver transplantation: age, gender, race, etiology of liver disease, additional comorbidity, smoking status, MELD score, serum creatinine, serum sodium (hyponatremia; serum sodium <135 mM), presence of ascites, and need for renal replacement therapy. Post-transplantation the following complications were documented only if occurring during the five yr follow-up period: perioperative renal replacement therapy, number of acute rejection episodes, chronic rejection, disease recurrence, and re-transplantation. The presence of diabetes, and diagnosed hypertension and dyslipidemia were noted if present during the same time period. All blood pressures, body mass index (BMI) measurements, calcineurin inhibitor trough levels, and blood lipid concentrations (non-fasting) taken during routine outpatient appointments were recorded. These were averaged to provide the average systolic blood pressure, average diastolic blood pressure, average BMI, average cyclosporine trough level, average tacrolimus trough level, average cholesterol concentration, and average triglyceride concentration. A comparable calcineurin trough level for the multivariate models was obtained for all patients regardless of calcineurin inhibitor by expressing the trough as relative to the median value. Obesity was defined as an average BMI greater than or equal to 30.

GFR was estimated using the Modification of Diet in Renal Disease (MDRD) Study 6-variable equation ($\text{eGFR} = 170 \times \text{creatinine (mg/dL)}^{-0.999} \times \text{age}^{-0.176} \times 1.180 \text{ (if black)} \times 0.762 \text{ (if female)} \times \text{serum urea nitrogen}^{-0.170} \times \text{albumin}^{0.138}$) (20). For each patient, the estimated GFR (eGFR) was calculated from the serum creatinine level taken at the time of listing for liver transplantation (pre-transplant), and from routine outpatient clinics at 0.5, 1, 2, 3, 4, and 5 yr post-transplantation. As per the National Kidney Foundation, CKD was defined as a sustained eGFR of <60 mL/min/1.73 m²: stage 3, stage 4, and stage 5 CKD were defined as an eGFR of 30–59 mL/min/1.73 m², 15–29 mL/min/1.73 m², and <15 mL/min/1.73 m² or on dialysis, respectively (4).

Statistical analyses

Normally distributed continuous variables were compared using the Student's *t*-test and nonparametric continuous variables the Mann-Whitney test. For each patient, the annualized change in eGFR was determined using simple linear regression of all eGFR measurements available from six months post-transplantation (17). A negative annualized change in eGFR represents a decline in eGFR, and a positive annualized change in eGFR represents an improvement in eGFR. The rate of change in eGFR expected with aging is 1 mL/min/1.73 m² per year (21). Therefore, patients were described as having a decline in renal function greater than the rate expected with aging if the annualized change in eGFR was >−1.00 mL/min/1.73 m² per year. Patients with an annualized change in eGFR of between −1.00 and +1.00 mL/min/1.73 m² per year and those with an annualized increase in eGFR of >+1.00 mL/min/1.73 m² per year were considered to have unchanged renal function and improved renal function, respectively (22).

Logistic regression analyses were used to examine the relationship between the annualized change in eGFR, and a decline in eGFR greater than the rate expected with aging, and the development of stage 3–5 CKD. Two separate multivariate models were constructed with all clinically relevant variables entered simultaneously. Pre-transplant hypertension was not included in the models because of small patient numbers. Multivariate linear regression analyses were then used to determine variables associated with the annualized change in eGFR. Again two multivariate models were constructed with all clinically relevant variables entered simultaneously. In model 2, the binary variables, insulin-dependent diabetes, diagnosed hypertension, and diagnosed dyslipidemia were replaced with the continuous variables, average glucose, average systolic blood pressure, and average cholesterol in an effort to examine whether modification of these factors might influence outcome. Age was not included in the models because of collinearity. Finally, logistic regression analysis was repeated to identify variables associated with a decline in renal function greater than the rate expected with aging. *p* < 0.05 was considered statistically significant at all times. Data were analyzed using the SPSS 18 package (SPSS Inc., Chicago, IL, USA).

The annualized change in eGFR is expressed as the mean with 95% confidence intervals (95% CI). All other values are expressed as mean and

standard deviation (SD), and median and interquartile range (IQR) as appropriate.

Results

Baseline characteristics of the patients at the time of listing for liver transplantation are outlined in Table 1. The median time from listing to transplantation was 39 (range 2–314) d.

Pre-transplant renal function

Pre-transplant, the mean eGFR of the entire cohort was 103 (SD 34) mL/min/1.73 m², and the median serum creatinine was 81 (IQR 70–97) μM. Three patients (3.1%) had an eGFR 30–59 mL/min/1.73 m², one patient (1.0%) had an eGFR 15–29 mL/min/1.73 m², and 0 patients had an eGFR <15 mL/min/1.73 m² (Fig. 1). Seventeen patients (17.5%) had refractory ascites, and 23 (23.7%) had hyponatremia.

Table 1. Clinical characteristics of patients at time of listing for transplantation

Clinical characteristics	
Age (yr)	50.9 (10.3)
Male:Female	1:1.2
Caucasian	93 (95.9)
Liver disease etiology	
Primary biliary cirrhosis	35 (36.1)
Alcoholic liver disease	21 (21.6)
Sclerosing cholangitis	11 (11.3)
Cryptogenic cirrhosis	8 (8.2)
Hepatitis C	7 (7.2)
Autoimmune hepatitis	5 (5.1)
Hepatitis B	4 (4.1)
Other	6 (6.2)
Hepatocellular carcinoma	7 (7.2)
MELD score	16 (5)
Measure of renal function	
Creatinine (μM)	81 (70–97)
eGFR (mL/min/1.73 m ²)	103 (34)
Sodium (mM)	138 (135–140)
Hepatorenal syndrome	2 (2.1)
Renal replacement therapy	0
Ascites	59 (60.8)
Refractory ascites	17 (17.5)
Comorbidity	
Diagnosed hypertension	6 (6.2)
Diagnosed dyslipidemia	3 (3.1)
Diabetes mellitus	12 (12.4)
Insulin-dependent diabetes	8 (8.2)
Active smoker	20 (20.6)
Obesity (wet weight)	18 (18.8)

MELD, model for end-stage liver disease; eGFR, estimated glomerular filtration rate.

Values expressed as mean (standard deviation), median (interquartile range), and number (percent) where appropriate.

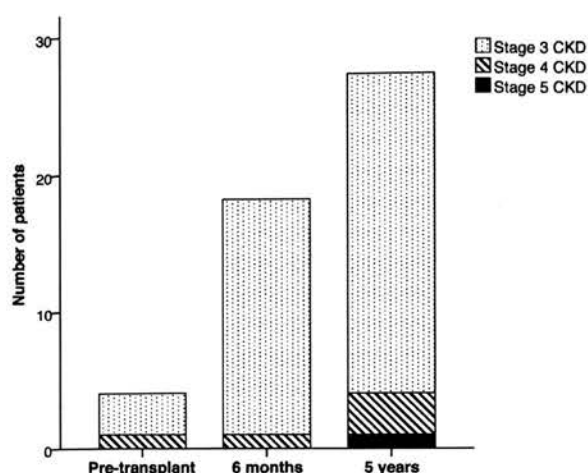


Fig. 1. Prevalence of chronic kidney disease pre-liver transplantation, and at six months and five yr post-liver transplantation.

Change in renal function following transplantation

Eight patients (8.2%) required renal replacement therapy during the immediate postoperative period.

By six months after transplantation, the mean eGFR was 75 (SD 22) mL/min/1.73 m², and the median serum creatinine was 103 (IQR 92–123) µM. Seventeen patients (17.5%) had stage 3 CKD, one patient (1.0%) had stage 4 CKD, and no patient had stage 5 CKD at this time point (Fig. 1).

During subsequent years, there was a progressive increase in the prevalence of renal dysfunction. At five yr post-transplant, 23.7%, 3.1%, and 1.0% of patients had stage 3, stage 4, and stage 5 CKD, respectively (Fig. 1). The mean five-yr eGFR was 69 (SD 21) mL/min/1.73 m², and the median five yr serum creatinine was 110 (IQR 98–129) µM.

eGFR declined at a mean rate of 1.08 mL/min/1.73 m² per year (95% CI 2.13–0.03, $p = 0.045$) from six months post-transplant. Forty-seven patients (48.5%) demonstrated a decline in renal function greater than the rate expected with aging. Twenty patients (20.6%) had no change in renal function, and 30 (30.9%) had an improvement in renal function. Assuming the mean annualized change in eGFR remained constant, the estimated prevalence of stage 3, stage 4, and stage 5 CKD by 10 yr post-transplant was 29.9%, 6.2% and 6.2%, respectively.

Change in eGFR as a predictor of CKD by five yr post-transplant

In patients who developed CKD, the mean rate of decline in eGFR from six months post-transplant was 2.50 (95% CI –4.03 to –0.97) mL/min/

1.73 m² per year compared with 0.52 (95% CI –1.35 to 0.30) mL/min/1.73 m² per year for patients who did not ($p = 0.016$). Sixty-seven percent of the CKD group demonstrated a decline in renal function greater than the rate expected with aging during the preceding years compared with only 41.4% of the non-CKD group ($p = 0.026$).

The annualized change in eGFR from six months after transplantation remained an independent predictor of five-yr CKD in a multivariate model including all clinically relevant variables simultaneously (Table 2, $p = 0.001$). Here, the more negative the change in eGFR (i.e., the greater the decline in eGFR), the greater the likelihood of CKD. In a similar multivariate model, a decline in renal function greater than the rate expected with aging was associated with a relative risk of CKD of 6.88 (95% CI 1.75–27.14, $p = 0.006$, adjusted for age, gender, hepatitis C status, pre-transplant eGFR, refractory ascites and diabetes, perioperative renal replacement therapy, and calcineurin inhibitor, data not shown).

Factors associated with the change in renal function from six months after liver transplantation

Given the strong association between the decline in renal function from six months post-transplant and the development of CKD statistical analyses were then performed to identify factors that may influence the long-term change in eGFR. Clinical characteristics of the cohort during the follow-up period are outlined in Table 3.

In a multivariate linear regression model adjusting for renal relevant variables, with the mean annualized change in eGFR as the dependent variable, a higher baseline eGFR ($p < 0.001$), female gender ($p = 0.006$), diagnosed hypertension ($p = 0.019$), and diagnosed dyslipidemia ($p = 0.034$) were associated with a faster rate of decline in renal function (Table 4, multivariate model 1). There was no association between the presence of hepatitis C infection, insulin-dependent diabetes or immunosuppression, and change in eGFR. In a second multivariate model, in which binary variables were replaced with continuous, average systolic blood pressure was strongly associated with the change in renal function: for every 10 mmHg increase in average systolic blood pressure, there was a 0.76 mL/min/1.73 m² per year faster decline in eGFR ($p = 0.005$, Table 4, multivariate model 2).

Logistic regression analysis was then used to determine variables associated with a rate of decline in renal function greater than that expected with aging (Table 5). In this multivariate model

Table 2. Univariate and multivariate logistic regression analyses of variables predictive of CKD by five yr after liver transplantation

Variable	Univariate		Multivariate	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age (yr)	1.07 (1.02–1.12)	0.011	1.09 (1.00–1.18)	0.043
Female gender	7.67 (2.40–24.52)	0.001	32.11 (3.00–343.71)	0.004
Hepatitis C	2.06 (0.43–9.90)	0.366	31.21 (1.41–688.86)	0.029
Pre-transplant eGFR (mL/min/1.73 m ²)	0.97 (0.95–0.99)	0.002	0.96 (0.93–0.99)	0.011
Refractory ascites	0.76 (0.23–2.59)	0.663	1.90 (0.21–17.32)	0.571
Diabetes mellitus	0.85 (0.21–3.40)	0.815	5.23 (0.30–91.05)	0.257
Perioperative RRT	5.08 (1.12–22.98)	0.035	33.40 (3.63–307.05)	0.002
CNI: cyclosporine	1.99 (0.78–5.07)	0.151	2.13 (0.51–8.96)	0.302
ΔeGFR (mL/min/1.73 m ² per yr)	0.86 (0.76–0.98)	0.021	0.63 (0.47–0.84)	0.001

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor; ΔeGFR, mean annualized change in eGFR from six months to five yr post-transplant. Reference group (relative risk 1.00): male gender, no hepatitis C, no refractory ascites, no diabetes mellitus, no perioperative RRT, CNI: tacrolimus. Bold-face indicates statistical significance.

after adjusting for all clinically relevant variables, a higher six month eGFR ($p < 0.001$), female gender ($p = 0.024$), and diagnosed hypertension ($p = 0.050$) were independent risk factors. There was a trend toward an association between cyclosporine and decline in renal function ($p = 0.071$).

Table 3. Clinical characteristics of patients after liver transplantation

Clinical characteristics	
Graft dysfunction	
Early acute cellular rejection	33 (34.0)
Late acute cellular rejection	8 (8.2)
Chronic rejection	4 (4.1)
Re-transplant	6 (6.2)
Immunosuppression	
CNI: cyclosporine	29 (29.9)
One month cyclosporine trough	146 (50)
One month tacrolimus trough	8.1 (3.0)
Comorbidity	
Diagnosed hypertension	46 (47.4)
Diagnosed dyslipidemia	10 (10.3)
Diabetes mellitus	21 (21.6)
Insulin-dependent diabetes	13 (13.4)
Obesity	33 (34.0)
Average value	
Cyclosporine trough (mM)	137 (20)
Tacrolimus trough (μg/L)	7.7 (1.3)
Systolic blood pressure (mmHg)	142 (15)
Diastolic blood pressure (mmHg)	82 (7.1)
Glucose (mM)	5.8 (5.2–6.9)
Cholesterol (mM, n = 91)	5.0 (1.3)
Triglyceride (mM, n = 51)	1.6 (1.3–2.7)
Body mass index	27.8 (5.1)

CNI, calcineurin inhibitor. Values expressed as mean (standard deviation), median (interquartile range), and number (percent) where appropriate.

Discussion

In this single-center study, we have described, for the first time, the annualized change in eGFR following liver transplantation. In a homogeneous cohort of patients with long-term survival, we examined the change in eGFR beyond the initial postoperative period, and current focus of CKD prevention. By estimating the steady-state change in kidney function, our aim was to identify possible modifiable risk factors for the progression to CKD. We have shown that liver transplant recipients had a clinically relevant decline in eGFR from six months post-transplant. Our patients demonstrated a mean decline in eGFR of 1.1 mL/min/1.73 m² per year, and almost half had a decline in renal function greater than the rate expected with aging. A decline in eGFR was a strong and independent predictor for the development of CKD. Multivariate modeling found a higher baseline eGFR, female gender, hypertension, and dyslipidemia to be associated with a faster rate of decline in renal function.

Several factors have been identified that may influence the development of chronic renal dysfunction following liver transplantation. Increasing age, white race, hepatitis C, pre-transplant diabetes mellitus, pre-transplant renal impairment, and perioperative acute renal failure are independent predictors of CKD (1,3,7,9,23). These variables highlight, at the time of surgery, the patients at increased risk of renal injury in whom preventative measures may be more crucial. However, few are potentially modifiable, and little

Late decline in eGFR after liver transplant

Table 4. Multivariate linear regression analyses of variables associated with the annualized change in eGFR from six months post-liver transplantation

Variable	Multivariate model 1			Multivariate model 2		
	B (95% CI)	β	p Value	B (95% CI)	β	p Value
Six-month eGFR (mL/min/1.73 m ²)	-0.108 (-0.141, -0.075)	-0.650	<0.001	-0.109 (-0.145, -0.073)	-0.680	<0.001
Female gender	-2.080 (-3.550, -0.609)	-0.284	0.006	-1.360 (-2.856, 0.137)	-0.192	0.074
Hepatitis C	-0.183 (-2.667, 2.300)	-0.013	0.884	-1.072 (-4.233, 2.089)	-0.069	0.502
Perioperative RRT	-0.480 (-2.761, 1.800)	-0.036	0.676	-0.118 (-2.602, 2.366)	-0.009	0.925
CNI: cyclosporine	-0.637 (-2.033, 0.759)	-0.080	0.676	-0.820 (-2.273, 0.632)	-0.106	0.264
Average CNI trough	1.447 (-2.397, 5.291)	0.063	0.456	-0.149 (-4.324, 4.026)	-0.007	0.944
Insulin-dependent diabetes	-0.559 (-2.484, 1.365)	-0.052	0.565			
Diagnosed hypertension	-1.523 (-2.788, -0.258)	-0.208	0.019			
Diagnosed dyslipidemia	-2.300 (-4.420, -0.179)	-0.192	0.034			
Log average glucose (mM)				-0.322 (-3.195, 2.531)	-0.025	0.818
Average systolic blood pressure (mmHg)				-0.076 (-0.128, -0.024)	-0.319	0.005
Average cholesterol (mM)				-0.033 (-0.641, 0.574)	-0.012	0.913

eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor.

Reference group (relative risk 1.00): male gender, no hepatitis C, no perioperative RRT, CNI: tacrolimus, no insulin-dependent diabetes, no diagnosed hypertension, no diagnosed dyslipidemia. Boldface indicates statistical significance.

Table 5. Univariate and multivariate logistic regression analysis of variables associated with a rate of decline in renal function from six months post-liver transplantation greater than the rate expected with aging

Variable	Univariate		Multivariate	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Six-month eGFR (mL/min/1.73 m ²)	1.03 (1.01–1.06)	0.002	1.06 (1.03–1.10)	<0.001
Female gender	1.47 (0.66–3.29)	0.345	3.94 (1.20–12.95)	0.024
Hepatitis C	0.40 (0.07–2.17)	0.288	0.878 (0.11–7.27)	0.904
Perioperative RRT	0.61 (0.14–2.72)	0.521	1.44 (0.23–8.84)	0.697
CNI: cyclosporine	3.37 (1.34–8.51)	0.010	2.68 (0.92–7.79)	0.071
Average CNI trough	0.832 (0.07–10.05)	0.885	1.10 (0.06–20.44)	0.949
Insulin-dependent diabetes	0.89 (0.28–2.90)	0.859	0.86 (0.19–3.86)	0.842
Diagnosed hypertension	1.86 (0.83–4.16)	0.133	2.68 (1.00–7.19)	0.050
Diagnosed dyslipidemia	1.68 (0.44–6.39)	0.444	2.95 (0.56–15.43)	0.200

eGFR, estimated glomerular filtration rate; RRT, renal replacement therapy; CNI, calcineurin inhibitor.

Reference group (relative risk 1.00): male gender, no hepatitis C, no perioperative RRT, CNI: tacrolimus, no insulin-dependent diabetes, no diagnosed hypertension, no dyslipidemia. Boldface indicates statistical significance.

information has been gained regarding appropriate strategies to delay or avoid the progression to CKD in high risk individuals. In two of three large randomized controlled trials of unselected patients, delayed perioperative administration of tacrolimus did not impact on renal function by one yr post-transplant (13–15). All three studies were marred by failure to maintain low tacrolimus trough levels, and Boudjema et al.'s trial has confirmed that long-term reduced calcineurin inhibitor exposure is probably more important (24). Reduction or withdrawal of calcineurin inhibitor therapy once CKD has developed results in only a marginal improvement in kidney function (10, 25–29). Therefore, prevention of CKD in liver transplant patients should be a priority (10).

The key outcome of our study was the observation that hypertension and dyslipidemia were associated with the rate of decline in renal function after liver transplantation. Echoing findings in non-transplant patients with CKD and the general population for every increment increase in average systolic blood pressure, there was a faster rate of decline in GFR (17, 30, 31). This suggests that tight blood pressure control may be of particular importance in limiting the progression to CKD. Diagnosed dyslipidemia was an independent predictor of change in eGFR, although we were unable to demonstrate a correlation with lipid levels. The diagnosis of dyslipidemia was made by primary care or non-transplant hospital physicians, and lipid levels were non-fasting and variably

performed in the study cohort. Consequently, the reported lipid levels are probably a poor reflection of the true lipid profile. In the non-transplant setting, the association between dyslipidemia and a faster rate of progression of kidney disease is contentious (17). However, many studies in patients with and without renal dysfunction have linked high cholesterol, triglyceride and LDL levels, and low HDL levels, with the rate of change in GFR. Furthermore, lipid-lowering therapy has been shown to slow the decline in renal function (17, 31, 32).

In our study, female patients had a faster rate of decline in eGFR than men, mirroring the gender difference demonstrated by renal transplant recipients (22). Female gender is a risk factor for chronic renal dysfunction following liver, heart, and lung transplantation (1). In contrast, in non-transplant patients male gender is linked with a faster rate of decline in GFR (17). A possible explanation for this discrepancy is that women may be more susceptible to calcineurin inhibitor-mediated renal injury.

The strong association between baseline eGFR and change in eGFR may represent a statistical phenomenon. Nevertheless, we attempted to minimize "regression to the mean" by estimating eGFR slope using a large number of measurements and by adjusting all analyses for baseline renal function (33, 34). We speculate that patients who had a higher eGFR at six months post-transplant had greater calcineurin inhibitor exposure than those with renal dysfunction. Moreover, additional potential contributing factors such as hypertension and dyslipidemia may have been monitored less closely in this group.

The retrospective nature of our study and relatively small patient numbers may explain the failure to demonstrate a relationship between diabetes, and immunosuppression, and change in eGFR (17). Furthermore, we did not have urate levels or urinary protein concentrations available on a sufficient number of patients to allow analysis (35). On the other hand, an advantage of the population size and methodology was that it allowed the close observation of risk factors over a prolonged time period. Additional potential limitations were the precision of the main outcome measures eGFR and eGFR slope. The former has been shown to be a less precise measure of GFR in liver transplant recipients than in other groups (36). However, it is the most widely accepted readily available measure of renal function. The precision of change in eGFR determined from the slope of eGFR over time increases with the duration of follow-up (33). All our patients had change in

eGFR calculated from six eGFR measurements and a prolonged follow-up time of five yr.

In conclusion, our study has shown for the first time that liver transplant recipients have a clinically relevant decline in eGFR from six months following transplantation. We have identified modifiable factors that may influence the change in eGFR and increase the risk of progression to CKD. Our study emphasizes the multifactorial nature of renal dysfunction following liver transplantation. Prospective studies are required to examine the effects of aggressive blood pressure and lipid control on the development of CKD in liver transplant patients.

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